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(57) Abstract

A method of improving the quality of a body of water (e.g. a lentic or slow moving body) comprises providing a pH modifying agent in the water. The agent is preferably CO_2 and the method is particularly effective for the treatment or prevention of blue-green algal growth.

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WATER TREATMENT

The present invention relates to the treatment of water to improve the quality thereof. The invention relates particularly, but by no means exclusively, to the treatment of standing (e.g. lentic) and slow moving bodies of water such as ponds, lagoons, lakes and reservoirs.

If the quality of water in a body of water is poor then health problems may result from contact with, and/or ingestion of, even a small amount of water. This is, of course, particularly disadvantageous in that bodies of water which might otherwise be used for recreational purposes etc. (e.g. swimming, windsurfing) cannot be so used if the water quality is too poor. Furthermore, precautions may need to be taken against the possibility of someone inadvertently falling in the water, e.g. by the provision of fencing which may be expensive.

One (but not the only) cause of poor water quality is the presence in the water of blue-green algae (cyanobacteria) which have come to the forefront of public awareness in recent years because of reported pet and livestock mortalities and human stomach and skin disorders in recreational water users (e.g. canoeists and windsurfers). These problems occurred as a result of ingesting or coming into contact with large numbers of blue-green algae which are being reported more and more frequently in reservoirs and lakes. Powerful hepato-toxins and neuro-toxins which can give rise to such problems have been isolated from several species of blue-green algae which occur in British waters. The potential toxicity of blue-green algae has resulted in restrictions being imposed on usage in many hundreds of water bodies throughout the U.K., which contained these algae during 1990. Out of a total of 915 water bodies surveyed by the National Rivers Authority during 1990, 594 contained blue-green algae. The problem of blue-green algae is not, or course, confined to the United Kingdom and occurs in many other countries.

Many blue-greens have the tendency to grow in large numbers in the water column forming "blooms" which colour the water green (giving a "pea soup" appearance) or form scums on the surface. Irrespective of whether or not these are toxic, problems may still arise during the treatment of potable waters producing poor tasting

and odorous drinking water and causing blocking of filters which increases the cost of treatment. Water based recreation and potable water treatment industries have a great desire, therefore, to control the growth of blue-green algae in many water bodies throughout the U.K and elsewhere.

To date no clearly defined factor has been identified which triggers the growth of these algae. Many factors have been suggested, including high water temperature, ability to survive at low light intensity, appropriate nitrogen and phosphorus concentrations, ability to avoid zooplankton grazing and ability to move through the water column by means of a buoyancy mechanism. A combination of some or all of these factors may be important at any one time. This has made successful control strategies difficult to determine. Reduction of nutrients, although desirable, does not always work and is often not possible. Grazing and light are difficult to manipulate in large bodies of water especially in the long term. Manipulation of buoyancy and turbulence has been attempted, sometimes with success, by using destratification and aeration devices. There have been many occasions, however, when these measures have been unsuccessful and have actually promoted blue-green blooms.

US-A-3 756 220 discloses a system for inhibiting the growth of algae and bacteria in chlorine disinfected swimming pools in which water from the pool is circulated around a line into which the combustion gases from a heater are introduced. Carbon dioxide and carbon monoxide present in these combustion gases are dissolved in the circulating water to produce a weak solution of carbonic acid which is constantly fed into the pool. The rate of addition of the weak acid to the pool may be adjusted to maintain the pH of the water in the pool in the range of 6.0 to 7.6 which is said to be the most suitable range for inhibiting the growth of algae and bacteria. Control of growth by the manipulation of pH under conditions prevailing in a swimming pool is more likely to reflect an increase in active hypochlorous acid induced by a reduction in pH (i.e. at the lower pH there is a greater percentage of active hypochlorous acid). Chlorine is a more effective biocide in the form of undissociated hypochlorous acid rather than in the dissociated form. The inhibiting effect is therefore likely to be due to the direct biocidal effect of

hypochlorous acid formed due to the abundance of chlorine present in the swimming pool.

There is no specific disclosure in US-A-3 756 220 as to the control of blue green algae. Moreover the system described in US-A-3 756 220 is applicable primarily to domestic or commercial swimming pools for which the heater (for generating the combustion gases) will either already be available or may readily be provided.

It is an object of the present invention to provide a method of improving water quality which is simple and effective to operate.

In its broadest aspect, the present invention provides a method of improving the quality of water comprising providing in the water a pH modifying agent (e.g. a gas such as a purified industrial gas) in an amount sufficient to effect a desired pH modification in the water.

The invention is based on the realisation that certain aspects of the quality of water are either directly or indirectly pH dependent, and that an appropriate adjustment of pH may conveniently be made by the use of a pH modifying agent provided in the water.

In a more specific aspect the invention provided a method of treating or preventing blue-green algal infestation in a body of water, the method comprising treating said body of water with a pH modifying agent to reduce the pH of said body of water.

The pH modifying agent used in the method of the invention may be a solid, liquid or gas (e.g. a purified industrial gas). Most preferably the pH modifying agent is carbon dioxide.

The method of the invention is therefore particularly applicable to the treatment of bodies of water which are prone to infestation by blue green algae. Examples of blue-green algal infestations which may be treated by the method of the invention include infestations by Oscillatoria, Microcystis and Anabaena.

The method of the invention is particularly effective for the treatment of water bodies having a volume of at least 50 m^3. The invention is most preferably applied to standing (lentic) or slow moving bodies of water, i.e. bodies of water which are not generally subject to turbulent flow.

The invention is applicable particularly to "natural" systems, i.e. systems in which the conditions prevailing are dictated by the natural environment.

The invention is therefore particularly applicable to open-air lentic or slow moving bodies, e.g. ponds, lakes, lagoons, reservoirs, dock basins, marinas, canals. The invention is also applicable to other water bodies e.g. water storage tanks. The pH modifying agent may be supplied to the water by an appropriate delivery system (examples of which will be given below) at a rate and for a time which is commensurate with the volume of the body of water being treated and the required pH. For example, the pH modifying agent (e.g. carbon dioxide) may be used for treating a body of water having a pH of 7 to 11 (e.g. 8 to 11) to one having a significantly lesser pH (e.g. 6 to 7 or 6.0 to 6.5, or 6.5 to 7, or 7 to 7.5).

The use of carbon dioxide to increase the acidity of water in this way is particularly effective in the control, or prevention, of growths of blue-green algae.

The carbon dioxide is preferably supplied to the body of water as a gas.

The method of the invention is most effective when the pH modifying agent is introduced into the body of water during a time when conditions therein are such as to be capable of supporting active growth of blue-green algal cells. Ideally therefore the treatment is applied when the blue-green algal cells are undergoing active growth.

The most suitable time for the introduction of the pH modifying agent is during spring and summer, the normal algal growth periods dictated by light and temperature levels. This also corresponds with the desire to apply the technique at the time before algal densities have reached their highest levels so making overall treatment more efficient and cost effective. Thus treatment during the spring, before blue-green algal populations become established is desirable for financial reasons. This prophylactic approach could minimise the quantities of the agent required to effect control. However, treatment at the onset of blooms even if in autumn will be effective.

Furthermore active algal growth requires sufficient availability of nutrients in the appropriate ratios. In laboratory experiments active algal growth was stimulated in cells that were not undergoing active growth by the addition of nitrates and phosphates (e.g. to levels of 35 mg/litre and 2.5 mg/litre in a ratio of 14:1). The presence

of similar nutrient regimes is thought to be required for active growth and so will be necessary for effective control by application of the pH modifying agent. The provision of an adequate level of nutrients in suitable proportions may reflect the requirement of algal cells for nutrients in photosynthetic pathways.

The most preferred pH modifying agent for use in the invention is carbon dioxide, preferably supplied to the body of water as a gas.

The carbon dioxide addition seems to increase the likelihood of deleterious viral infections of Oscillatoria cells. There is also some evidence that carbon dioxide interferes with normal intracellular activity thus damaging the cell or inhibiting (or altering) cell physiology. Intracellular changes within the algae may be precipitated by the addition of carbon dioxide and result in senescence and death of the algae.

In addition to the apparent effects upon cellular physiology and the likelihood of viral infection, intracellular effects of CO_2 addition may include the precipitation of calcium carbonate (as in the generation of limestone in marine environments). This process of biomineralisation or more specifically calcification, is known to occur in cyanobacteria during periods of active growth under higher light levels and/or temperatures possibly associated with seasonal and more long term climatic changes. This may relate to the appearance of granular structures within the cells revealed by our experiments. This in turn could be used as a measure of the efficacy of a control process, or the state of condition of an algal population. The process of biomineralisation may reflect a significant diversion of energy or resources which might contribute to a reduction in algal growth or productivity.

Preferably the amount of carbon dioxide supplied to the water body is such as to give a concentration of 5-20 ppm expressed as free CO_2 in the water. The optimum amount will vary depending on the physico-chemical characteristics (e.g. conductivity, hardness and alkalinity) and the maintenance of the carbon dioxide as free CO_2. The aforementioned physico-chemical parameters can of course vary with time and between separate water bodies.

The method of the invention using carbon dioxide relies upon controlling the carbon supply to the algae. It has long been assumed

that carbon, an essential element for life, is present in adequate amounts in virtually all waters. The chemical species and hence the availability of carbon varies however with pH. It is predominantly in the form of free CO_2 below pH 6.5, as bicarbonate at pH 8 and as carbonate above pH 9. Blue-greens tend to dominate in waters which are alkaline. In these waters free CO_2 is very low and bicarbonates/carbonates are high. It is believed that low CO_2/high pH favours blue-greens but higher CO_2/lower pH favours other species such as green algae (all other things being equal). If the latter conditions prevail, the indications are that blue-green algae and their attendant problems will be eliminated. Other algal groups, such as the non-toxic green algae may then predominate. These are much more acceptable in ecological and aesthetic terms (e.g. they are more easily controlled by natural grazing by zooplankton) and are not considered a nuisance to the same extent as blue-greens. In addition they are often more easily dealt with from a water treatment viewpoint, an important consideration for reservoir management. Moreover, the effect of CO_2 injection is to reduce overall algal biomass. This is an important effect which will have implications for the water industry who have an interest in removing total algal material by physical filtration.

The use of CO_2 injection to control blue-green algae is a more realistic proposition than adjustment of pH by addition of large amounts of acids into water bodies which may be unacceptable. By using currently available aeration/oxygen injection/diffusion devices, it is possible to both increase the free CO_2 and lower the pH by injecting the required amounts of CO_2 into the water column.

For the treatment, or prevention of, growths of blue-green algae by provision of carbon dioxide in the water, it is preferred that the pH of the water is adjusted to a value of 6-8. The most effective pH will be in the range of 6-7.5 but for economic reasons (based on the cost of carbon dioxide) it may be preferred to operate at a pH of 6.5-7.5.

The delivery of the CO_2 (or other pH modifying gas) into the water may be effected using injection/diffusion equipment already known in the art for aerating bodies of water. The delivery system should promote a degree of mixing so that substantially uniform pH

conditions are achieved throughout at least the portion of the body of water which is to be treated (e.g. the photic zone). Delivery systems which may be used for the invention include venturi-type injection systems (e.g. as available from BOC under the name Vitox) as well as air lift systems and diaphragm diffusers. It is however also within the context of the present invention simply to pass the gas along a pipe or other conduit provided in the water, the pipe having gas discharge outlets from which the gas may be released at an appropriate rate into the water being treated.

The most preferred delivery systems are those which result in substantial dissolution of the pH modifying gas into the water to ensure that the gas is not simply lost by bubbling upwards through the water. The Vitox unit is particularly suitable for ensuring such gas dissolution.

Irrespective of the delivery system used, the pH modifying gas may be delivered as the sole gas or in admixture with another gas or gases, e.g. air.

Generally the delivery system will be provided either on the bed or within the water column of the body of water being treated. The number and positioning of the delivery system(s) used for a particular application will depend on the area and depth of the body of water to be treated, as well as on the pH change which is to be achieved, but may readily be determined by those skilled in the art.

It will be appreciated that the method of the invention may be operated continuously or intermittently. Continuous operation will generally be effected where it is desired to maintain the water at a substantially constant pH. Intermittent operation will generally be effected when it is acceptable to maintain pH within a particular range, in which case, once a pH value at the extreme of the range has been achieved by use of the method, the treatment may be discontinued until such time as the pH moves towards the other end of the range or until the algae re-appear.

It will be further appreciated that a precursor of the pH modifying gas, rather than the gas <u>per se</u>, may be delivered to the water. Thus, for example, in the case of carbon dioxide, it is possible to introduce the gas in the form of solid carbon dioxide, particularly if steps can be taken to ensure dissolution of the carbon dioxide

rather than simple floating of the solid material to the water surface. Alternatively, an aqueous solution of carbon dioxide (particularly a supersaturated solution) may be used.

The invention will be illustrated by the following Examples and accompanying drawings which illustrate the result of the Examples.

Example 1

Materials and Methods

Samples were collected from two field locations both of which supported rigorous growths of blue-green algae. Salford Quays, which are enclosed freshwater docks, have almost continuous dense growths of Oscillatoria. Rosthern Mere (near Knutsford) is a wetland of international importance, a nature reserve and is highly eutrophic supporting bloom proportion growths of Microcystis.

Twelve 3 litre flasks were arranged in two groups of 6 in the laboratory. The flasks of the first group of 6 were kept as natural Salford Quays water (2l in each flask) and are identified below as Q_n (where n is 1-6). The second group of 6 were Salford Quays water with added nutrients (nitrogen and phosphorus) and are identified below as Q_nN. Three pairs of flasks had CO_2 added, two pairs had air but no CO_2 and one pair had neither air nor CO_2. A similar series was arranged for the Rosthern Mere water with 2l/flask except only one pair had air added. The conditions for each flask are given in Table 1.

Table 1. Experimental Conditions in Flasks

Q_1N	Q_2N	Q_3N	Q_4N	Q_5N	Q_6N
M_1N	M_2N	M_3N	M_4N	M_5N	
High CO_2	Med.CO_2	Low CO_2	-	Low Air	High Air
+N&P	+N&P	+N&P	-	+N&P	+N&P

Q_n & M_n series as above but with no added nutrients.

Results

The results for the main experiment are shown in the

accompanying Figures 1 to 4. Though the experiment was repeated twice, the results were similar and only one set is presented.

Rosthern Mere:

For the first three pairs of bottles where CO_2 was added the mean pH value maintained were 5.4 (bottles M, M_1N), 5.6 (bottles M_2, M_2N) and 6.01 (bottles M_3, M_3N). Bottles M_4, M_4N, M_5 and M_5N had an average pH of 8.59.

In the series without added nutrients there was a general depression of growth which was more marked in the presence of CO_2. When nutrients were added only those with CO_2 showed a reduction, those without showed enhanced growth.

Salford Quays:

For the first three pairs of bottles where CO_2 was added the mean pH values were 5.5 (for bottles Q_1 & Q_1N), 5.9 (bottles Q_2 & Q_2N) and 6.1 (bottles Q_3 & Q_3N). Bottles $Q_{4,5,\& 6}$ and $QN_{4,5,\& 6}$ had an average pH of 8.54.

In the series without added nutrients there was a general, although not very marked, decline in growth with only a small difference between added CO_2 and CO_2-free samples. In the series where nutrients were added there was a more marked depression of growth where CO_2 was added and an overall increase with no CO_2.

Discussion

Both Salford Quays and Rosthern Mere were nutrient poor waters during the period when samples were collected. However, in these locations there is a degree of nutrient cycling from bottom deposits, decomposing plant and animal material inputs from rain and, in Rosthern, small inflows from streams. This cycling of nutrients was enough, in the natural systems, to sustain substantial growth in those waters. When a sample was enclosed in an experimental flask, however, apart from a small amount of recycling from decaying cells, no additional inputs of nutrients were possible so severe nutrient limitations resulted. The nutrient limitation was probably the overriding factor and when CO_2 was added in whatever quantities

there was only a small additional depression of growth.

When both nitrogen and phosphorus were added to either water, however, there was quite a marked increase in growth in three weeks except when CO_2 was added as well. In this latter case there was a depression in growth. This decrease in growth was most marked with Rosthern Mere (M series) suggesting that Microcystis responded more rapidly to the added CO_2. The effect was observed by day three in the high CO_2 flasks and by day seven in the lower CO_2 flasks with nutrients. In Salford Quays water there was only a slow decline in growth with no nutrients (over three weeks) and only a little faster (two weeks) when nutrients were added. This suggests that Oscillatoria responded more slowly to the added CO_2.

The overall growth, as indicated by chlorophyll a concentrations (units = µg/litre), does not give the whole picture however. Although chlorophyll levels remained high in the Q series there were indications of species changes in the populations. Green algae (mainly Scenedesmus and probably Ulothrix) were becoming increasingly common and naviculoid diatoms were also starting to colonise the glass surfaces. There was thus a more marked decline in Oscillatoria than was indicated by chlorophyll alone - the Chl a concentration being maintained because of species changes. Similar effects could be seen in the M series especially where nutrients were added when substantial growths of chlorococcalean green algae and pennate diatoms were observed towards the end of the experiments.

Conclusions

1. CO_2 additions rapidly decreased populations of the blue-green alga Microcystis.

2. Such reductions did not occur with air alone or with no gas at all.

3. If nutrient stress occurred this seemed to have an overriding influence.

4. In all flasks where growth was present at the end of the experiment green algae and diatoms were playing an increasingly

important role in the community.

5. CO_2 reduced growths of Oscillatoria but more slowly than with Microcystis.

6. Facts 2, 3 and 4 above also apply to the Q series flasks with Oscillatoria.

Example 2

II: Pilot Scale

Example I was extended to assess the effectiveness of CO_2 injection on other blue-green algal species. Further samples containing predominantly Microcystis aeruginosa and Anabaena flos-aquae were therefore included within this Example. These waters were sampled from Eccup Reservoir, Yorkshire. The Salford Quays water samples and the Eccup water samples differ markedly in hardness and pH. Each sample therefore required varying CO_2 dosage rates to manipulate availability of carbon to algae. Similarly, the quantities of CO_2 required to manipulate different species are uncertain but have important economical and practical implications for CO_2 injection on a larger scale in field trials. Consequently samples from each site were maintained at two separate pH bands during the experiment using different CO_2 dosage rates.

Methodology

Samples from both Salford Quays and Eccup were collected and transferred to 200 litre tanks in the laboratory. Each tank contained 150 litres of sample, a sufficient volume to allow sampling of water for biological and chemical analysis throughout the course of the experiment without significant loss of water. All tanks were exposed to uniform lighting and mixing conditions. Lighting regimes of 10 hours light/14 hours dark were imposed. Minimal mixing was employed so as to prevent the break up of colonial and algal rafting species but was maintained at sufficient levels to allow adequate circulation of CO_2. Two tanks from each source were controlled at a pH of between 6.0-6.5 and a further two tanks were maintained between pH

6.0-7.0. pH was monitored approximately hourly to record fluctuations and so allow suitable adjustment of CO_2 flow to each tank to maintain each tank within its respective pH band. A further tank from each source acted as a control with similar mixing and lighting regimes but without CO_2 injection.

Nutrients were added initially to all tanks to ensure an excess of Phosphates and Nitrates at the start of the experiment. Nutrients were subsequently monitored throughout the experiment to prevent nutrient limitation of algae.

Changes in algal abundance and composition and any corresponding changes in zooplankton abundance were monitored approximately every 5 days throughout the trial. Water samples from each tank were taken and analysed microscopically to identify the phytoplankton and zooplankton species present and estimate their densities. Chlorophyll extraction was performed on separate water samples to give an estimate of algal standing crop.

<u>Results</u>

Chlorophyll analysis results represent estimates of total chlorophyll that is the sum of chlorophyll a and degradation products of chlorophyll that may be present. Phytoplankton were identified and cell densities estimated. These results were then converted to biovolumes occupied by each species in order to give a more accurate estimate of biomass allowing for the considerable variation in volume between each phytoplankton species. Total biovolumes of blue-green algae, green algae, diatoms and cryptophytes were then calculated and then expressed as a proportion of the total algal biovolume. Zooplankton were identified individually to species level but to simplify the presentation and interpretation of results total zooplankton numbers are given.

<u>Eccup Reservoir</u>

Chlorophyll analysis

Throughout the experiment, chlorophyll estimates were generally higher in both of the treatments than the control (Fig 5). With the exception of one of the tanks in the pH 6.0-6.5 range, chlorophyll tended to increase throughout the duration of the experiment corresponding to increases in the proportion of greens and diatoms.

Phytoplankton composition and abundance

Figures 6, 7 and 8 represent changes in phytoplankton species and abundance throughout the experimental period in the treatments at each pH and the control. In the higher pH range rapid decreases in both Microcystis and Anabaena occurred (indicated by an arrow) followed by a relatively small increase in blue-greens, mainly Anabaena over the next 10-12 days. After this, blue-greens were virtually eradicated alongside a coincident limited increase in the biovolume of green algae and diatoms. In terms of absolute numbers, however, increases in greens and diatoms were considerable. The initial decrease in blue-greens was to some extent mirrored in the control but was followed by a sustained increase in both blue-greens and other algae during the remainder of the experimental period although a slight reduction was observed at the end of the period. The lower pH range samples contained a lower abundance of blue-greens at the start of the experiment although these also exhibited an early decrease (indicated by an arrow) followed by a greater subsequent increase during the subsequent 10-11 days, again largely dominated by Anabaena. By the end of the experimental period, however, the blue-greens had virtually disappeared, alongside the appearance of limited number of greens and diatoms.

Zooplankton abundance

Considerable variation in zooplankton numbers in the treatments and controls was observed at both pH ranges (Fig. 9). Patterns between treatments and controls were, therefore, difficult to detect. In general, however, zooplankton numbers increased during the experimental period. Notably the zooplankton samples from each tank were dominated initially by rotifers and eventually by cladocerans such as Cyclops and Daphnia towards the end of the period.

Salford Quays Samples

Chlorophyll analysis

Chlorophyll concentrations in both treatments and the control remained similar and constant throughout the early part of the period. During the last 10 days, however, gradual increases in chlorophyll were observed in both treatments and the control. The last sampling

occasion revealed a rapid increase in chlorophyll in the control as compared to the treatments, again in both pH ranges. This increase coincided closely with an increase in blue-green algal density observed in the control.

Phytoplankton composition and abundance

In the higher pH range a reduction in Oscillatoria numbers was achieved during the initial 16-20 days of the experimental period (Fig. 11). In one tank, however, a subsequent increase in blue-greens was observed at the end of the period (indicated by an arrow). This corresponded to the appearance of a blue-green algal species, Anabaena circinalis, which is prevalent when nutrients become limiting as it is capable of fixing nitrogen from the atmosphere which gives it a competitive advantage over other algal species. It would appear from this that nitrates became limiting toward the end of the experiment in this tank creating conditions conducive to a bloom in Anabaena circinalis. Reductions in Oscillatoria in tanks held at the lower pH range were less consistent (Fig.12). In one of the two tanks blue-greens were eventually reduced to low levels by the end of the period after some fluctuation throughout the experiment. The second tank exhibited a gradual increase during the initial 20 days of the experiment but this was followed by a considerable decrease on the last sampling occasion. The apparent increases in blue-greens in the treatment tanks were, nevertheless, relatively small compared to those seen in the control which also continued throughout the entire experimental period (Fig.13).

Green algae and diatoms appeared in relatively small amounts compared to blue-green algae when expressed as biovolumes. In terms of absolute numbers, however, increases in these algae towards the end of the period was considerable although they occupy a smaller volume and so represent a smaller biomass.

Zooplankton abundance

Zooplankton numbers remained relatively constant in the control throughout the entire period (Fig. 14). Greater fluctuations were observed in the treatments, particularly in the higher pH range which generally exhibited greater abundance than in the lower pH range. In

both ranges, however, zooplankton numbers increased throughout the study period.

Conclusions

Control of Oscillatoria, Microcystis and Anabaena to either very low levels or their complete disappearance was achieved at both pH ranges. In most cases the reduction in blue-greens was closely followed by a considerable increase in green algae and diatoms to very high densities although these only represented small increases in algal biovolume. Greens and diatoms occupy very small volumes relative to blue-greens and so this represents an overall reduction in algal biomass. It seems, therefore, that CO_2 injection results in an overall reduction of algal biomass. This was not detected by patterns of chlorophyll concentration as greens and diatoms tend to contain larger amounts of chlorophyll per cell than blue-greens and so the effect of decreasing blue-green algal biomass on chlorophyll concentration will have been masked by the increase in green algae, albeit an increase of lower biomass. Chlorophyll content also varies within cells on a diurnal basis. Consequently, it is a less reliable indicator of algal biomass than cell number or biovolume.

The replacement of blue-greens by greens and diatoms has important implications for water quality and treatment in addition to the problems created by the toxicity of blue-green algae. Water bodies dominated by blue-greens effectively contain a larger volume of algae than water dominated by greens and diatoms.

Throughout the experiment zooplankton numbers tended to increase in the treatments but not in the controls. This would seem to reflect the greater availability of green algae and diatoms, a food resource generally preferred by zooplankton to blue-greens.

It is uncertain whether greater or more rapid control of blue-greens was achieved at the lower pH range by injecting greater amounts of CO_2. Greater control of CO_2 injection through valved flow meters allowed the manipulation of pH to within discrete pH ranges. However, the system employed relies upon manual control of CO_2 through the valves. Water samples with different physical and chemical characteristics respond differently to injection of CO_2 and the response of a given water body will also vary diurnally, dependent

upon the relative photosynthetic and respiratory activity of the algae. In addition each tank eventually began to behave differently as the algal composition and abundance changed throughout the experimental period. It is extremely difficult and also very labour intensive to precisely control pH to the exact required levels without a pH monitoring system which initiates a feedback control upon the flow of CO_2 to regulate pH continuously.

In conclusion, blue-green algae can be controlled by the use of CO_2 injection. As predicted, environmentally favourable populations of green algae and diatoms are produced as a result. In addition, the resultant green algal and diatom populations represent a considerable reduction in biomass compared to the blue-green algal populations they replaced.

Example 3

FIELD TRIALS AT SALFORD QUAYS

Introduction

The field experiment was designed to see whether control of Oscillatoria could be implemented by the addition of CO_2, given the large increase in scale of the experiment when compared with the laboratory trials, the greater environmental heterogeneity, and also whether this would result in the replacement by other algal populations which are less of a nuisance. Effects upon the zooplankton community and any negative effects upon the existing, healthy zoobenthos and fish populations would also be investigated. The field trial also included an assessment of the impact of CO_2 injection upon diurnal activity of phytoplankton* and zooplankton.

Methodology

Two identical 1-acre basins at Salford Quays, a freshwater dock system subject to severe Oscillatoria blooms, were chosen for the field experiment. These are connected by a narrow canal which normally does not allow significant mixing between the two basins. A butyl rubber membrane was installed across the canal to isolate the two basins after the initial stages of the trial. This was completed by 18th September, effectively isolating the experimental basins from each

other and the remaining basins in the Quays. One basin (7a) was used as a control whilst the other (7b) received CO_2 gas at a rate sufficient to maintain the pH of the basin between 6.8 and 7.0. Successful control of Oscillatoria had already been achieved using this pH range in the laboratory. Both basins were subject to artificial mixing using a helixor* system which utilizes compressed air to mix water. The helixors maintain oxygen levels in the dock basins and do not negatively effect the Oscillatoria population.

Aided by the helixor system, CO_2 was distributed uniformly throughout the treatment basin. This system continued to operate effectively for the duration of the experiment.

Monitoring of surface and bottom algal populations was initially undertaken weekly but after one month the frequency of surface sampling was increased to approximately twice weekly. Chlorophyll* levels and zooplankton densities were monitored weekly, while macroinvertebrates* and fish were sampled monthly. Fish data was collected in the treatment and control basins for comparison during the trial. Because of the intermixing between these experimental basins during the initial stages of the trial and the removal of the isolation membrane after the trial was completed, basin 8 was used as a control for the fish survey. Physico-chemical measurements were made in parallel with biological parameters. Oxygen and temperature profiles were measured weekly as was light extinction using a Secchi disc*. The surface and bottom nutrient status of each basin was assessed by fortnightly measurement of water samples for nitrates, nitrites, phosphates, total nitrogen and total phosphorus.

RESULTS AND CONCLUSIONS

Control of Oscillatoria was partially successful during the first 30 days of the trial. Weather conditions caused unforeseen mixing between the control and treatment basins which seemed to upset this trend. Although blue-green algal production* in the treatment basin was still frequently lower than in the control basin, the considerable reductions seen prior to the mixing and subsequent isolation were not repeated on the same scale. CO_2 injection did, however, promote growth of other forms of algae during the latter part of the trial.

Nutrient data indicates that nitrate and phosphates were probably limiting. Their relative concentrations varied considerably, indicating that N:P ratios were likely to be sub-optimal for algal growth. The weather conditions for a large part of the trial did not favour algal growth either.

This information suggests that the failure to reproduce the level of control seen in laboratory trials may have been due to an overall lack of algal population growth during the trial period. Oscillatoria, like many blue green algae, are capable of periods when growth is suspended during poor environmental conditions. Carbon is an important nutrient required for algal growth. No carbon will be assimilated while growth is suspended, and consequently manipulation of the carbon availability will have mush less of an impact upon the Oscillatoria population. To investigate further the effects of nutrient availability and poor weather conditions on algal growth, laboratory-based investigations would be necessary.

Any changes in the phytoplankton population that may have occurred did not result in an obvious response by the zooplankton. This might have been expected as the zooplankton feed upon the phytoplankton and would be expected to increase as numbers of greens, diatoms and cryptophytes increased. Injection of CO_2 at these levels had no obvious effect upon fish and macroinvertebrate populations, and did not result in water quality that contravened the EC Fisheries Directive.

Example 4

FURTHER PILOT SCALE STUDIES

Introduction

Results of the field trial suggest that the variable effectiveness of the field trial might be the result of reduced physiological activity by the algae due to low light levels, reduced temperatures and nutrient deficiency in the experimental basins during the trial period. Thus it was decided to assess the impact of these factors upon algal abundance and composition by bringing samples into the laboratory from the study basins and subjecting them to a range of enhanced

environmental conditions, including nutrient addition. Samples from the treatment basin (7b) were taken alongside samples from basin 8. Basin 8 was considered a more suitable control for this investigation since the period of intermixing between basins 7a (the original control) and 7b will have exposed the algal populations in the original control to CO_2.

The physiological activity of the algae was directly assessed by measuring the rate of oxygen production by photosynthesis* in the laboratory. This gives an indirect measure of the photosynthetic rate of the algae to assimilate carbon and hence their growth capacity; from this information it is possible to determine whether the algae were in a suitable physiological state for CO_2 injection to be effective.

METHODOLOGY

The design of the laboratory trial was based on that of the pilot-scale studies used in Example 2. Samples collected from basins 7b and 8 at Salford Quays were transferred to 200 litre tanks in the laboratory. Each tank contained 150 litres of sample, a volume sufficient to allow sampling of water for biological and chemical analysis throughout the course of the experiment without significantly affecting the total volume. All tanks were exposed to uniform lighting and mixing conditions, nevertheless, samples were randomly assigned to tanks located underneath the lighting apparatus. Lighting regimes of 10 hours light/14 hours dark were imposed approximately reflecting the external environmental conditions. The pH range of 6.8-7.0 employed in the field trial was used in the laboratory trial. This was a pH range found to be effective in Example 2. CO_2 injection was controlled manually via flow meters attached to each tank and pH was monitored approximately hourly.

CO_2 and nutrient additions corresponded to the following design:

TANK SET	A	B	C	D
SOURCE BASIN	7A	8	7A	8
ADDITIONS	CO_2 & NUTRIENTS	NONE	CO_2 ONLY	NUTRIENTS ONLY
REPLICATES	3 TANKS	3 TANKS	3 TANKS	3 TANKS

Nitrates and phosphates were maintained at a ratio of 14:1 in the tanks where nutrients were added (A and D). This is a frequently applied ratio thought to be suitable for algal culture. Minimum phosphate concentrations of 2.5 $mg.l^{-1}$ were necessary to ensure a slight excess of phosphate. Nutrients were measured upon initial sampling from the Quays, and nitrates and phosphates were added to produce the required ratio at the correct concentration. Nutrients were monitored weekly in each tank throughout the trial and adjustments in concentration made in the appropriate tanks (A and D).

Changes in algal abundance and composition were monitored approximately every 5 days, while zooplankton densities were monitored every 10-14 days. Samples for chlorophyll analysis were taken every 5 days to give an estimate of algal standing crop. Corresponding changes in water clarity were also monitored using Secchi depth as a measure of light extinction.

Light/dark productivity experiments* were undertaken to estimate algal photosynthetic activity using algal samples from the experimental tanks which were sealed in bottles. This type of experiment in short comprised two samples taken from each tank; the dissolved oxygen concentration was measured directly in each, and then one bottle was darkened using silver foil. All bottles were then suspended in their appropriate tanks just below the water surface for 4 to 6 hours. The increase in dissolved oxygen in the light bottles represents net photosynthesis. The reduction in dissolved oxygen in the dark bottles represents gross respiration. The sum of gross respiration and net photosynthesis divided by the time of exposure gives the gross photosynthesis for a given volume of algae of a known density per unit time. This is an indirect measure of the rate of growth.

RESULTS

Chlorophyll Analysis and Water Clarity

Chlorophyll concentrations rose in all tanks immediately after samples were brought into the laboratory, reflecting the improvement in constancy and intensity of light and temperature (which ranged between 19-22°C)(Fig. 15). This effect was most marked in samples from

basin 8 after the addition of nutrients. A lesser response was also recorded in the samples from basin 8 which did not receive nutrients. Notably background levels of nutrient concentrations in basin 8 tend to be higher than in basin 7 which would explain the increased growth after enhancement of conditions initially in the laboratory irrespective of artificial nutrient addition. The addition of nutrients to samples from the treatment basin (7b) also caused an increase in chlorophyll concentration indicating a considerable capacity for growth which had been hitherto suppressed by a combination of poor weather conditions and nutrient limitation. Chlorophyll levels in samples which received nether CO_2 nor nutrients remained low throughout the trial.

A decline in chlorophyll level was seen in tanks shortly after the initial increases representing a reduction in total algal biomass. Only in basin 8 samples where nutrients had been added but where CO_2 was not did chlorophyll levels remain elevated above their original concentration, reflecting the need for nutrient addition. Where both CO_2 and nutrients were added together a decline in chlorophyll levels was observed toward the end of the trial. Chlorophyll did not disappear completely, however, because of the presence of greens, diatoms and cryptophytes in the tanks. Chlorophyll levels after addition of only nutrients were much higher as Oscillatoria growth remained unchecked by CO_2 addition. Nevertheless, these levels also declined, albeit more gradually. It is likely that at these high densities further growth is limited by competition between Oscillatoria individuals for nutrients and light.

Water clarity decreased rapidly from between 60-70 cm Secchi depth in the field to between 12-27 cm Secchi depth after 3 days in the laboratory. The addition of CO_2 however, rapidly improved the water clarity and the tanks cleared after 18 days (Secchi disc visible at bottom of tank). Slight improvements in clarity were also recorded in tanks that received neither nutrients nor CO_2 possible due to nutrient starvation; however Secchi depth levelled out between 30-40 cms toward the latter part of the trial. The tanks where nutrients alone were added did not show any improvement in water clarity after the increased turbidity of the first few days. Secchi depths for tanks receiving CO_2 would have been much greater had the tanks been deeper and this would certainly have demonstrated more clearly how

CO_2 effectively cleared the water of algae after nutrients had been added.

Phytoplankton Composition and Abundance

Oscillatoria volumes increased markedly in the tanks to which nutrients were added (Fig. 17a) by a factor of 1.4 after 7 days. Without the addition of nutrients Oscillatoria volumes remained fairly constant throughout the trial irrespective of CO_2 addition. CO_2 injection after nutrient addition, however, brought about a rapid reduction in Oscillatoria volumes compared to tanks without CO_2 injection but with nutrient addition - these continued to increase markedly (by a factor of 2.3 after 17 days compared to the initial biovolume) before reaching a plateau toward the end of the trial.

Amounts of greens, diatoms and cryptophytes were relatively small compared to the biovolumes of Oscillatoria measured during the trial (Fig. 17b). Plots of mean biovolumes of these algae for each tank set do not reveal consistent patterns largely because of the great variability between each replicate tank in each set. Although pilot scale tanks had 150 l this was a relatively small volume compared with the original Quays basin and, due to the original overwhelming dominance of the Oscillatoria population, it was a matter of chance whether other algae were present in the various experimental tanks at the start of the trial. No consistent pattern was apparent with respect to the addition of either CO_2 or nutrients. However, the presence of greens, diatoms and cryptophytes contributed to chlorophyll readings in tanks where Oscillatoria were almost eradicated. Consequently chlorophyll was still detected in these tanks, although at much lower levels.

Zooplankton Abundance

Zooplankton numbers increased in all tanks during the trial (Fig. 18). The addition of both CO_2 and nutrients had little effect upon mean population numbers. Biovolumes of green algae, diatoms and cryptophytes, the algae which zooplankton graze preferentially, exhibited a general reduction throughout the experiment in these tanks indicating that increased zooplankton densities must be attributable to factors other than increased food supply.

Light/Dark Productivity Estimates

Productivity measurements were made immediately after bringing the samples in from the field. These indicated that algal productivity was much greater in samples from the control (basin 8) site than the samples already treated with CO_2 in the field. In the basin 8 samples which received added nutrients, dissolved oxygen production was more than three times that of the remaining samples (Fig. 19). Initially production in basin 8 samples, which did not receive added nutrients, was approximately twice as great as the samples from the treatment basin, although productivity soon fell to levels similar to those found in samples from the treatment basin. Background nutrient levels are frequently higher in basin 8 than basin 7b which would stimulate this initial increase in productivity upon enhancement of conditions in the laboratory. Productivity in the remaining two sets of tanks, where CO_2 was added to samples from the treatment basin, remained relatively low. These results indicated that algal production and growth were being limited in the field due to a lack of nutrients in the basins.

CONCLUSIONS

Provision of improved light, temperature and nutrients compared with field conditions produced greater population growth and increased algal biomass.

Growth of Oscillatoria was controlled in all tanks where CO_2 was injected, control being the most effective in the presence of nutrients which initially supported Oscillatoria growth before CO_2 had an effect.

CO_2 injection resulted in a considerable decrease in algal biomass but not in its complete elimination because of the presence of green algae, diatoms and cryptophytes.

The rapid response of algal production in samples from the treatment basin after the addition of nutrients and the improvement in environmental conditions revealed the capacity for growth of Oscillatoria which had been suppressed in the field.

The effect of CO_2 injection is greatly reduced while algal production is suppressed and consequently manipulation of the form

of carbon that is available to the algae will not be effective. Therefore, only during active growth does Oscillatoria seem to be markedly susceptible to the injection of CO_2.

CLAIMS

1. A method of treating or preventing blue-green algal infestation in a body of water, the method comprising treating said body of water with a pH modifying agent to reduce the pH of said body of water.

2. A method as claimed in claim 1 wherein the body of water has a volume of at least 50 m^3.

3. A method as claimed in claim 1 or 2 wherein the pH modifying agent is introduced into the body of water during a time when conditions therein are such as to be capable of supporting active growth of blue-green algal cells.

4. A method as claimed in claim 3 wherein the pH modifying agent is introduced into the body of water during active growth of blue-green algal cells.

5. A method as claimed in any one of claims 1 to 4, wherein the pH modifying agent is a gas.

6. A method as claimed in any one of claims 1 to 4, wherein the pH modifying agent is carbon dioxide.

7. A method as claimed in claim 6 wherein the amount of carbon dioxide supplied to the water body is to give a concentration of 5-20 ppm expressed as free CO_2 in the water.

8. A method as claimed in claim 6 or 7 wherein the carbon dioxide is substantially all dissolved in the water.

9. A method as claimed in any one of claims 6 to 8 wherein the carbon dioxide is introduced into the water by a carbon dioxide supply arrangement positioned on the bed of the water body.

10. A method as claimed in any one of claims 6 to 8 wherein the carbon dioxide is introduced into the water by a carbon dioxide supply arrangement supported in the body of water with clearance

above the bed of the water body.

11. A method as claimed in any one of claims 6 to 10 wherein the carbon dioxide is delivered into the water by a delivery system which promotes a degree of mixing to provide a substantially uniform pH throughout that portion of the body of water to be treated.

12. A method as claimed in any one of claims 6 to 11 wherein the carbon dioxide is supplied to the body of water as a gas.

13. A method as claimed in any one of claims 6 to 12 wherein the carbon dioxide is delivered into the water by a venturi-type injection system.

14. A method as claimed in any one of claims 6 to 12 wherein the carbon dioxide is delivered into the water by an air lift system.

15. A method as claimed in any one of claims 6 to 12 wherein the carbon dioxide is delivered into the water by a diaphragm diffuser.

16. A method as claimed in any one of claims 6 to 15 wherein the carbon dioxide is supplied to the water in admixture with air.

17. A method as claimed in any one of claims 6 to 11 wherein the carbon dioxide is delivered to the water body as an aqueous solution.

18. A method as claimed in any one of claims 1 to 17 wherein the initial pH of the body of water is in the range 7 to 11.

19. A method as claimed in claim 18 wherein the treatment is effected to reduce the pH of the water to 7.5 or below.

20. A method as claimed in claim 18 wherein the pH is reduced to 6.0 to 7.0.

21. A method as claimed in claim 20 wherein the pH is reduced to 6.0 to 6.5.

22. A method as claimed in claim 20 wherein the pH is reduced to 6.5 to 7.0.

23. A method as claimed in claim 19 wherein the pH is reduced to 7.0 to 7.5.

24. A method as claimed in any one of claims 1 to 23 wherein the body of water is a standing (lentic) body of water or slow moving body of water.

25. A method as claimed in claim 24 wherein the body of water is a pond, lake, lagoon, dock basin, reservoir, marina, canal, or is in a tank.

26. A method of treating or preventing blue-green algal growth in a body of water having a volume of at least 50 m^3 and normally having a pH above 7 comprising treating said body of water with carbon dioxide to reduce the pH of the body of water.

27. A method of improving the quality of water comprising providing in the water a pH modifying agent in an amount sufficient to effect a desired pH modification in the water.

28. A method as claimed in claim 27 wherein the pH modifying agent is a gas.

29. A method as claimed in claim 27 wherein the pH modifying agent is a liquid.

SALFORD QUAYS – NO NUTRIENTS

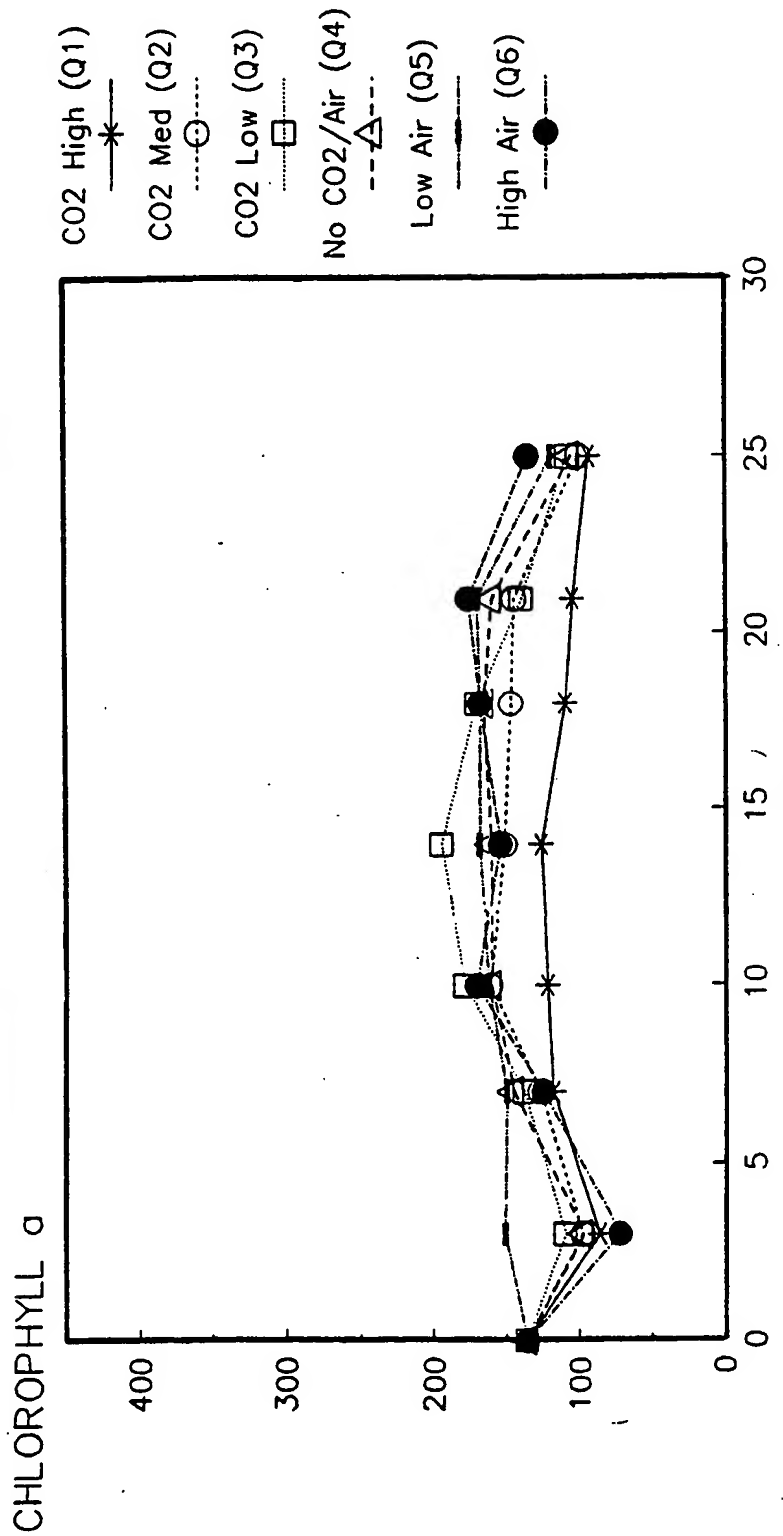


FIG.1

SALFORD QUAYS – NUTRIENTS

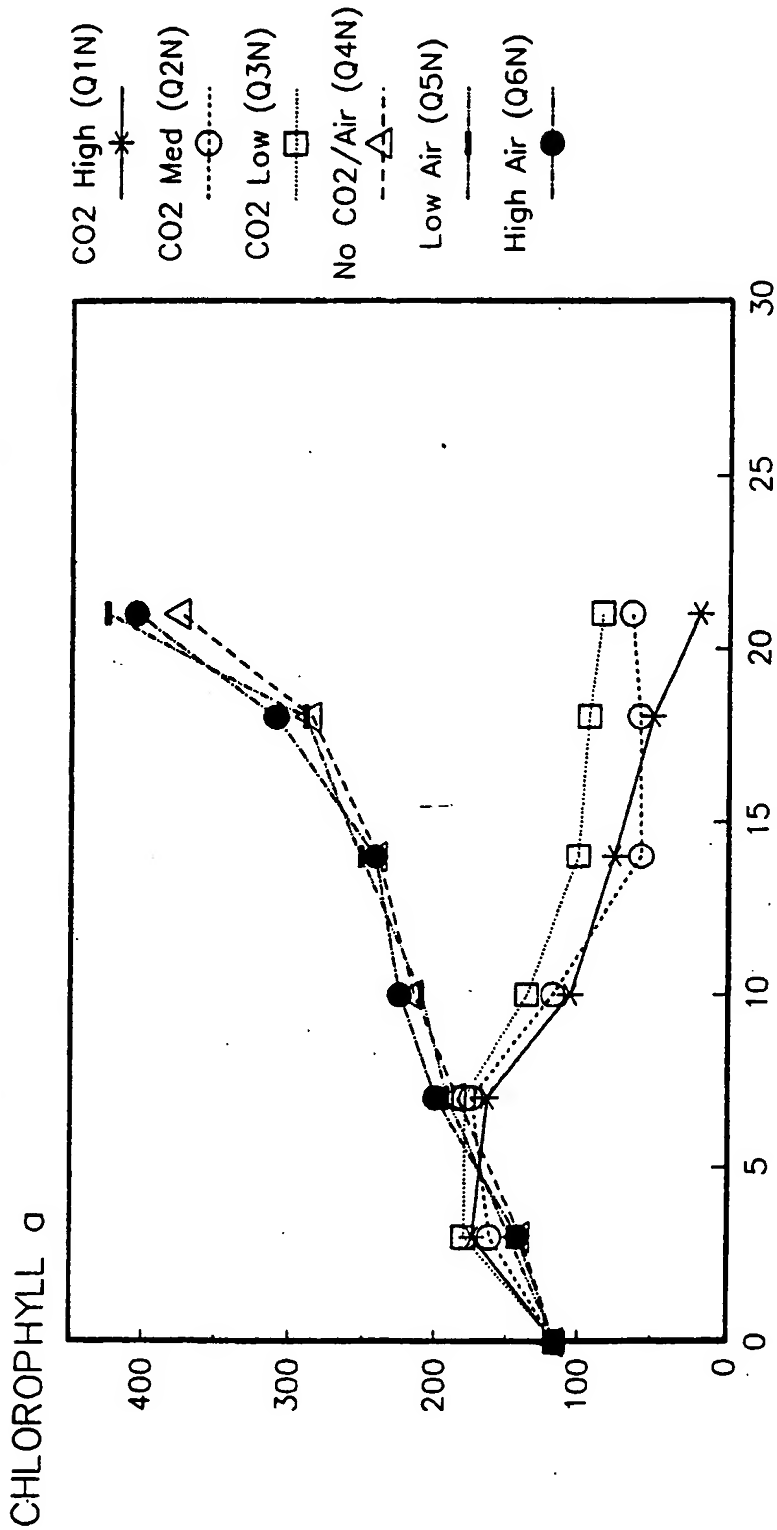


FIG. 2

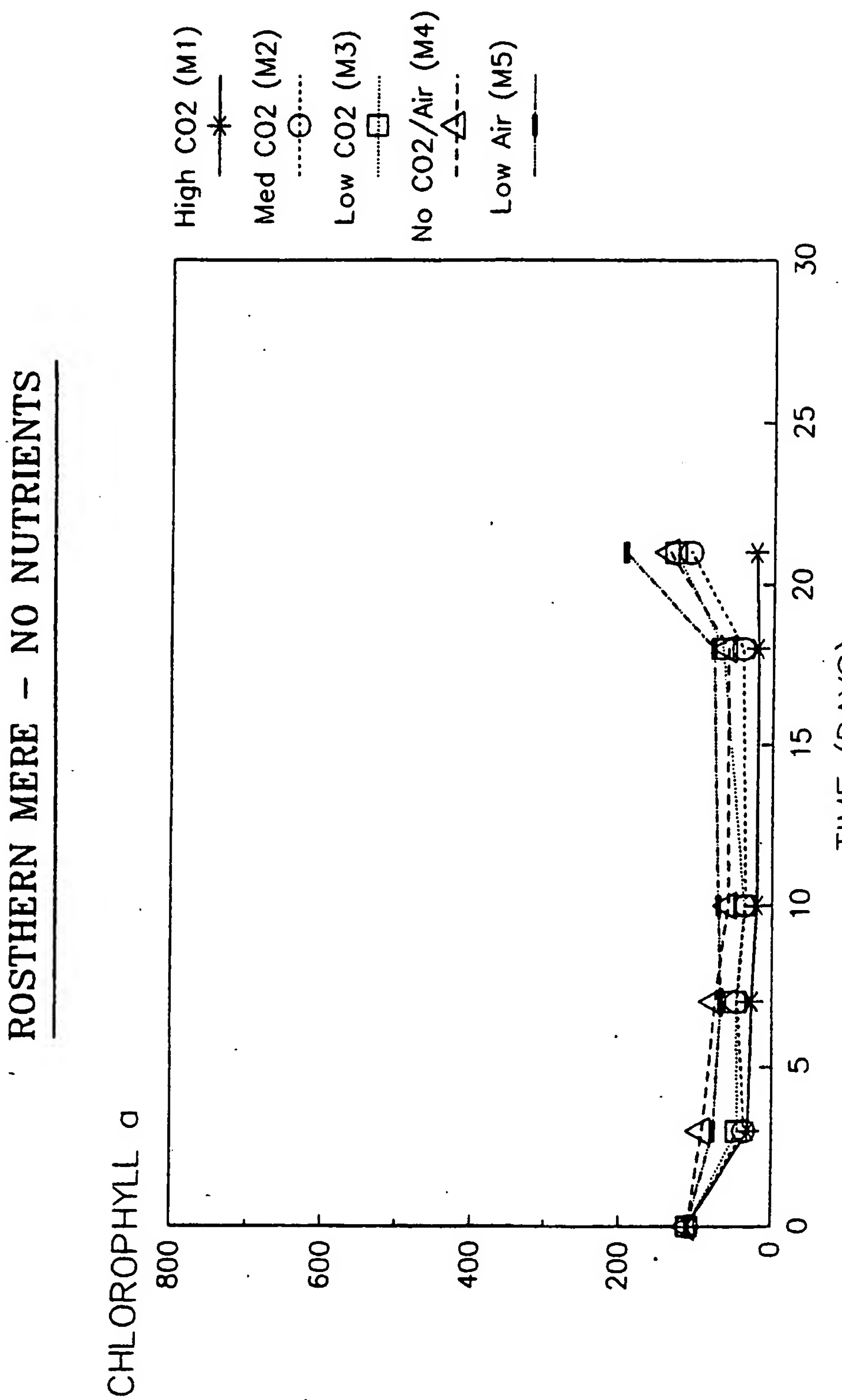

ROSTHERN MERE – NO NUTRIENTS
CHLOROPHYLL a
800
600
400
200
0
0
5
10
15
20
25
30
TIME (DAYS)
High CO2 (M1)
Med CO2 (M2)
Low CO2 (M3)
No CO2/Air (M4)
Low Air (M5)

FIG. 3

ROSTHERN MERE - NUTRIENTS

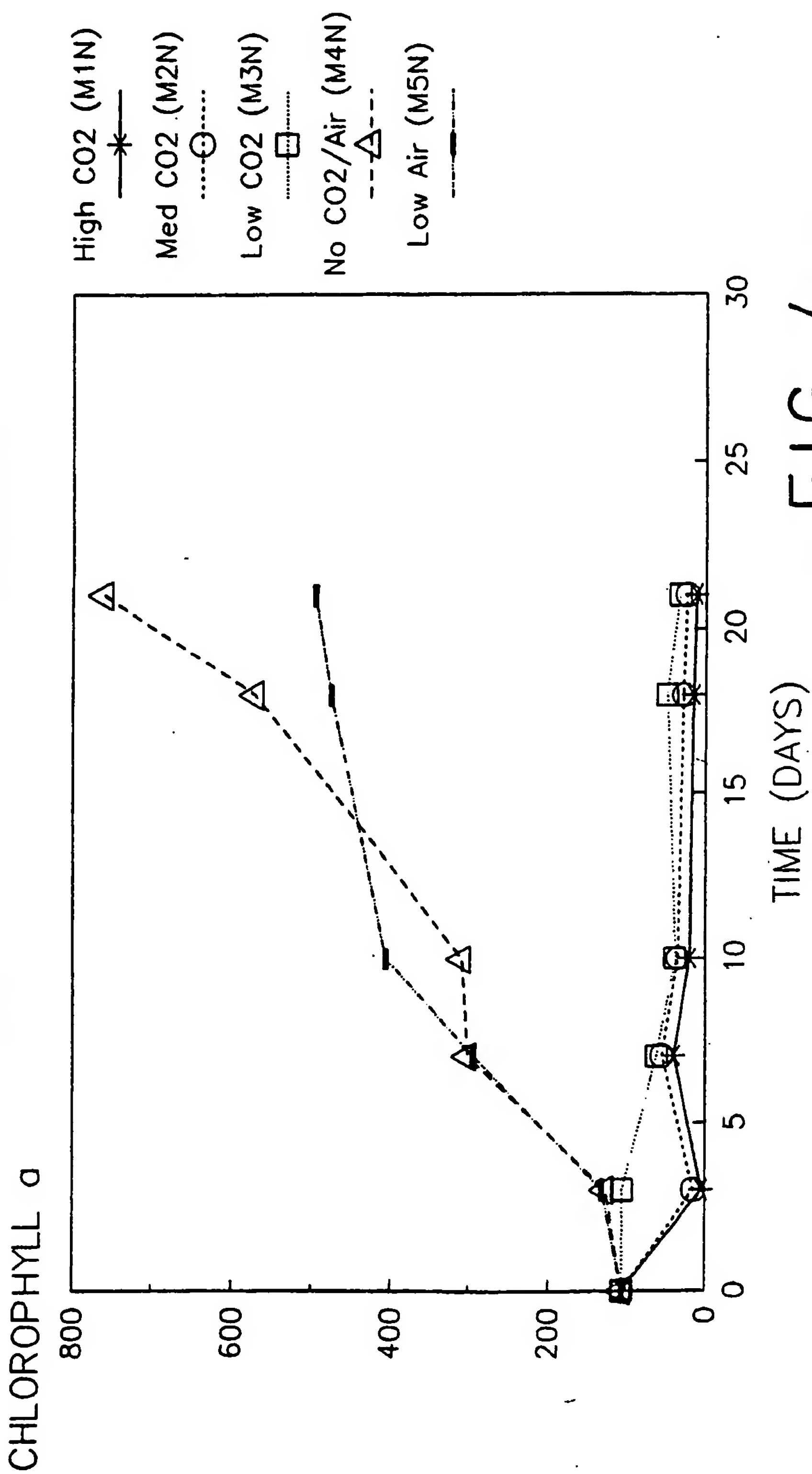
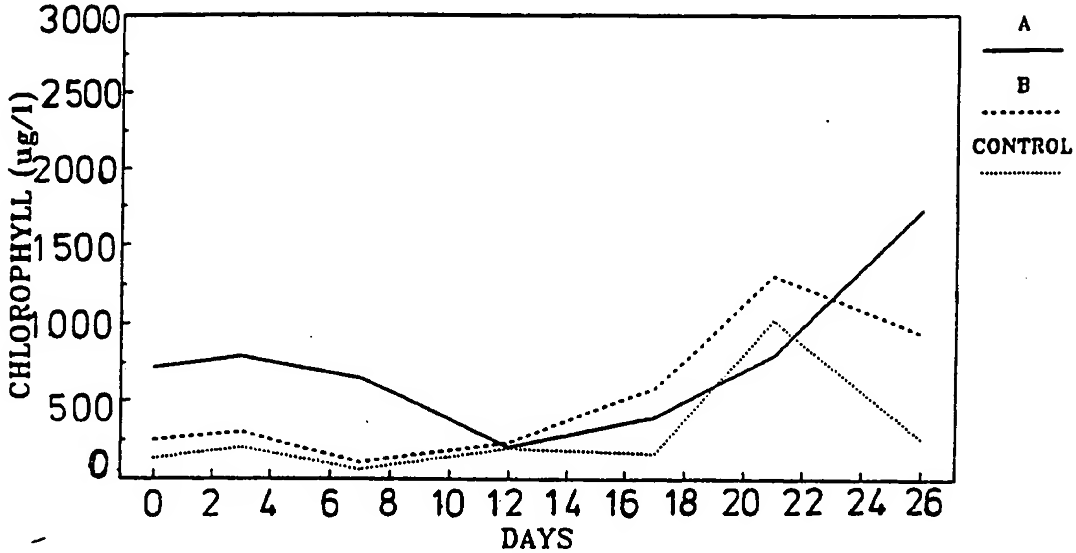


FIG. 4

TOTAL CHLOROPHYLL
ECCUP RESERVOIR - MICROCYSTIS/ANABAENA
pH RANGE: 6.5 - 7.0



pH RANGE: 6.0 - 6.5

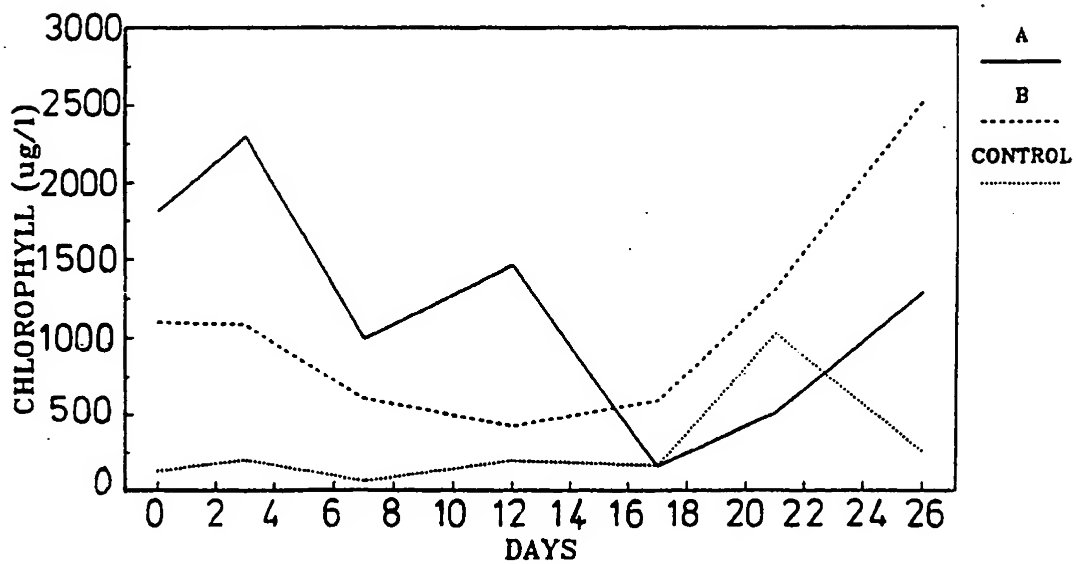
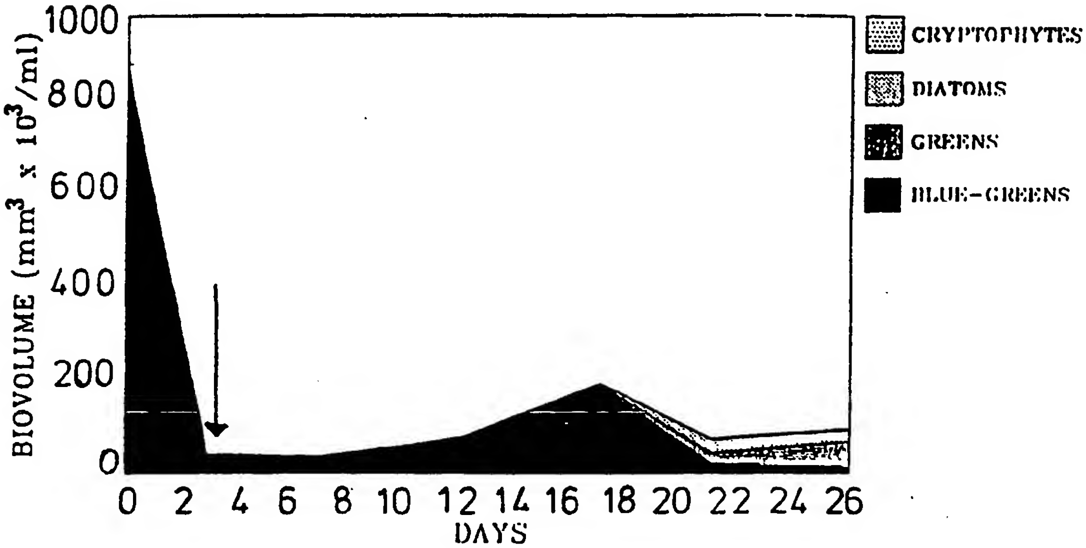


FIG. 5

PHYTOPLANKTON
ECCUP RESERVOIR - MICROCYSTIS/ANABAENA
pH RANGE: 6.5 - 7.0



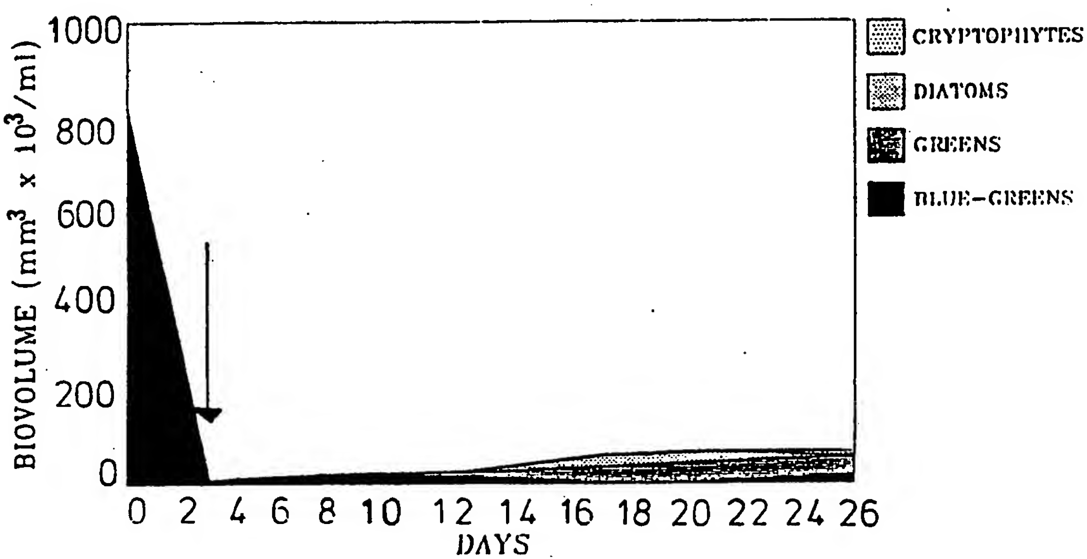


FIG. 6

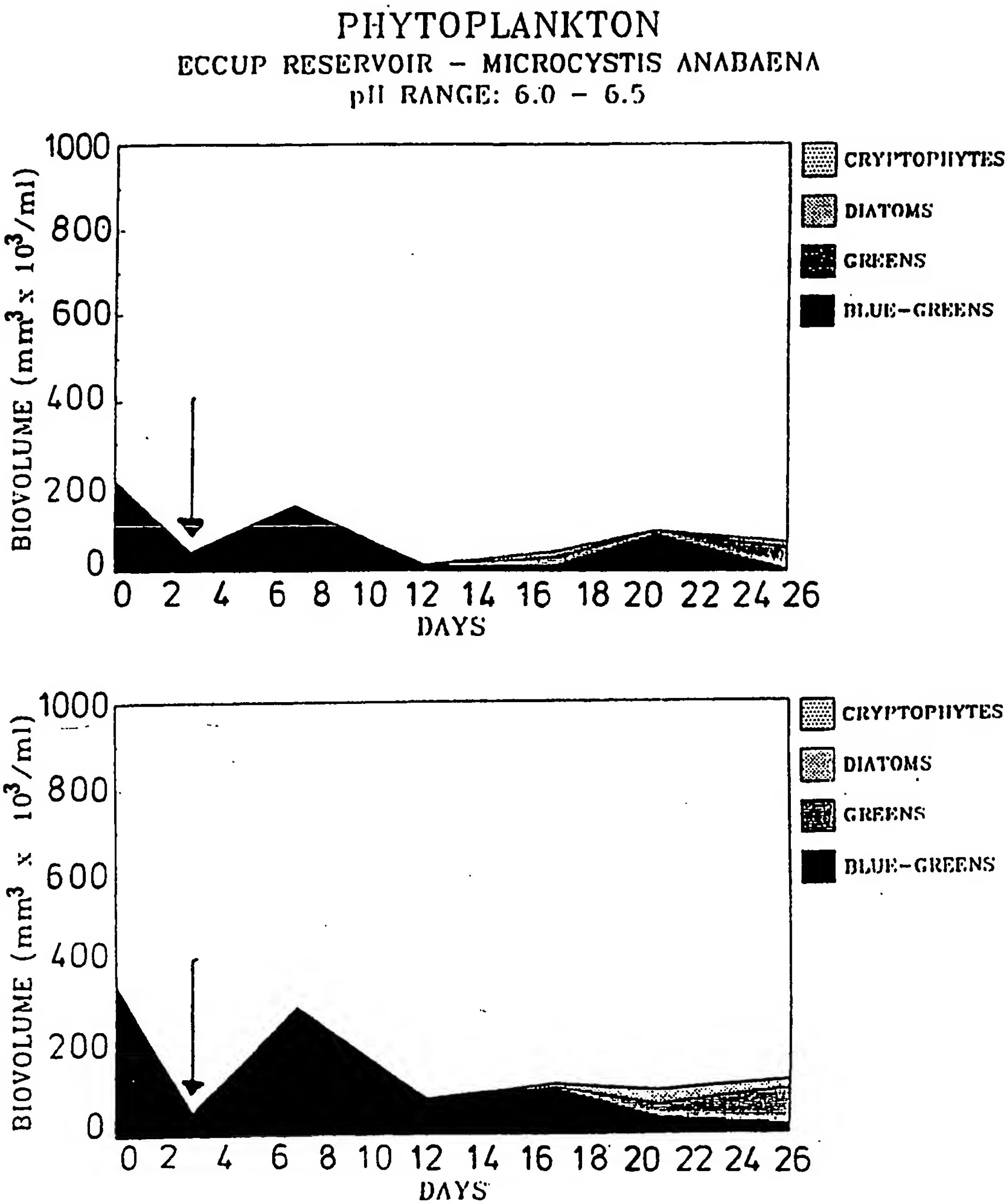

PHYTOPLANKTON
ECCUP RESERVOIR - MICROCYSTIS ANABAENA
pH RANGE: 6.0 - 6.5
BIOVOLUME (mm³ x 10³/ml)
1000
800
600
400
200
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
DAYS
CRYPTOPHYTES
DIATOMS
GREENS
BLUE-GREENS
BIOVOLUME (mm³ x 10³/ml)
1000
800
600
400
200
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
DAYS
CRYPTOPHYTES
DIATOMS
GREENS
BLUE-GREENS

FIG. 7

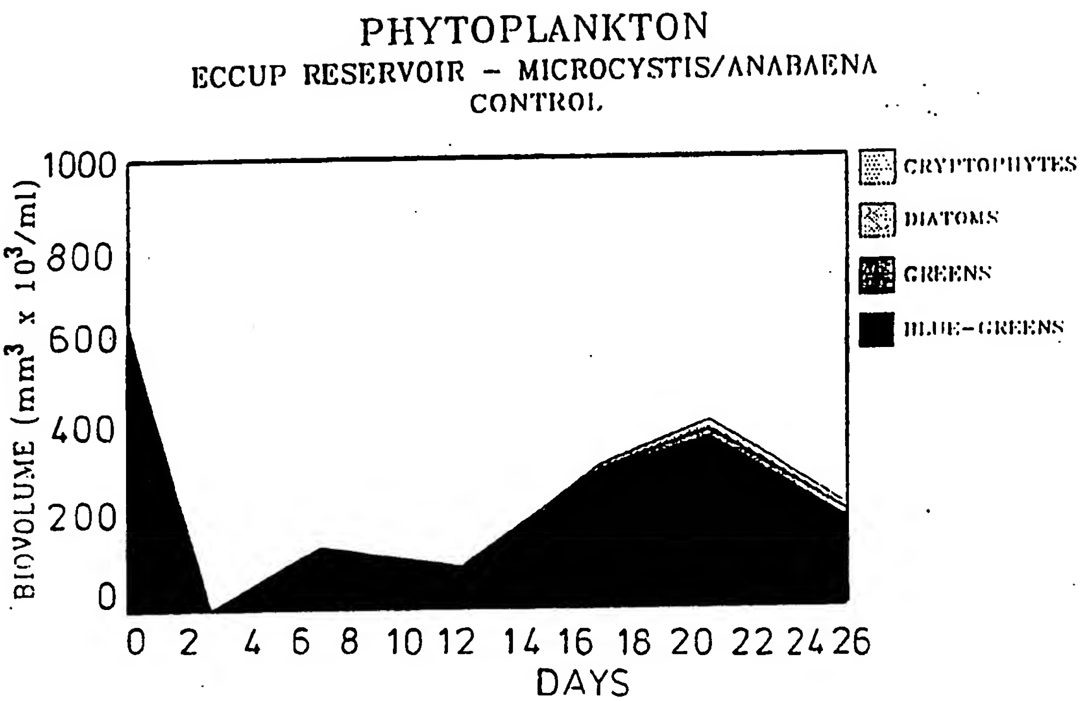
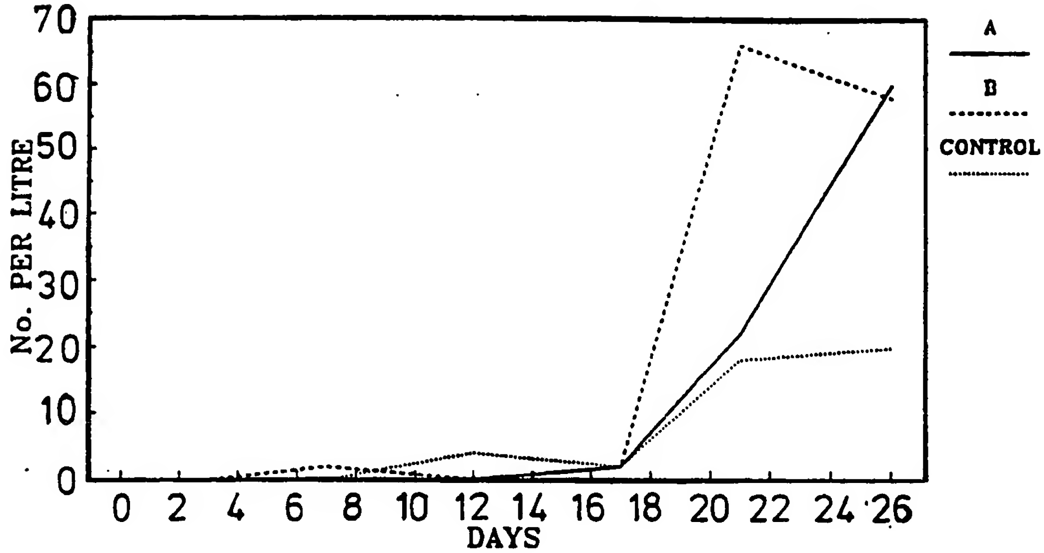

PHYTOPLANKTON
ECCUP RESERVOIR - MICROCYSTIS/ANABAENA
CONTROL
BIOVOLUME (mm³ x 10³/ml)
1000
800
600
400
200
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
DAYS
CRYPTOPHYTES
DIATOMS
GREENS
BLUE-GREENS

FIG. 8

ZOOPLANKTON

ECCUP RESERVOIR - MICROCYSTIS/ANABAENA

pH RANGE: 6.5 - 7.0



pH RANGE: 6.0 - 6.5

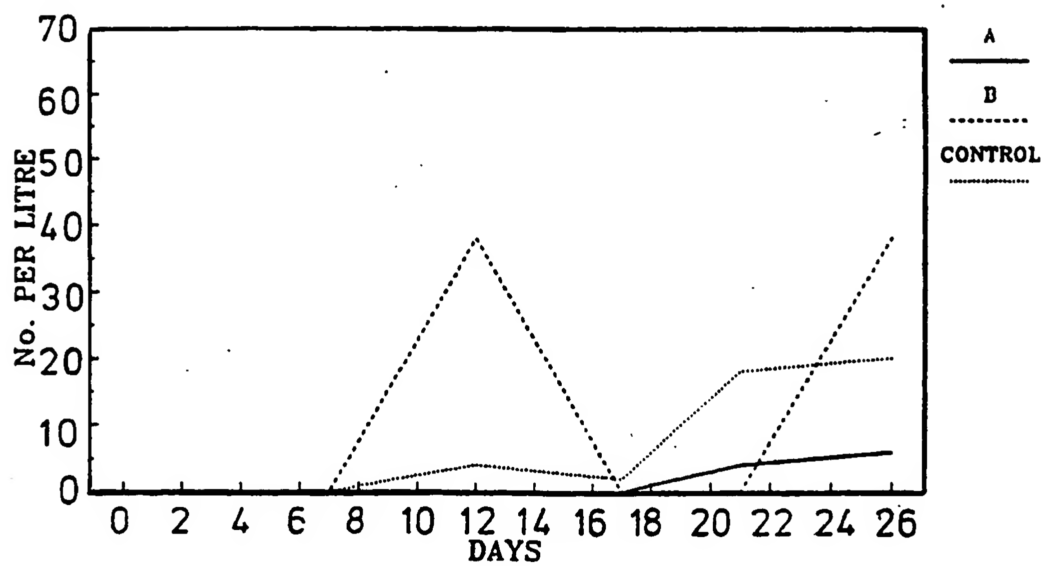
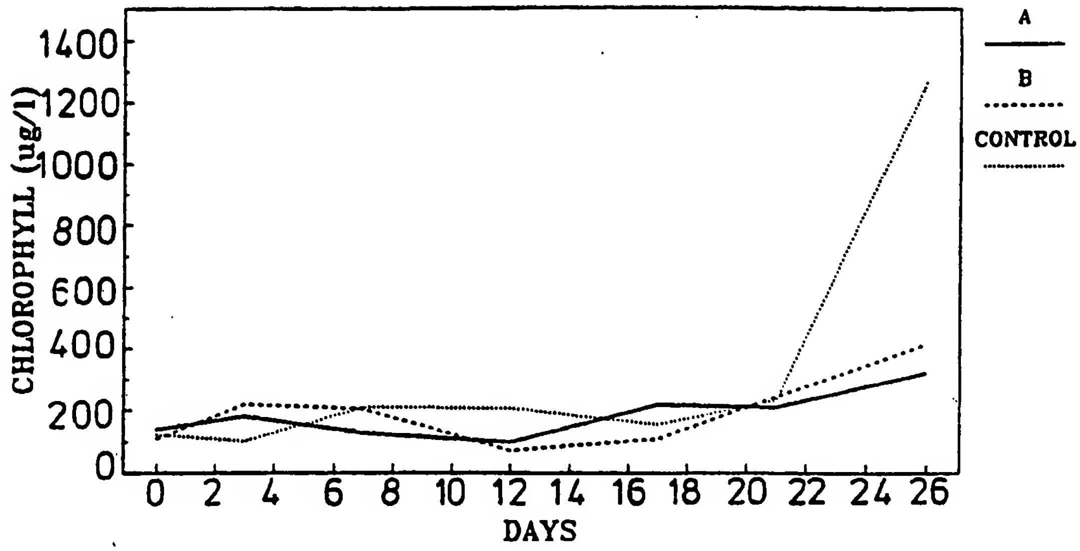


FIG. 9

TOTAL CHLOROPHYLL
SALFORD QUAYS – OSCILLATORIA
pH RANGE: 6.5 – 7.0



pH RANGE: 6.0 – 6.5

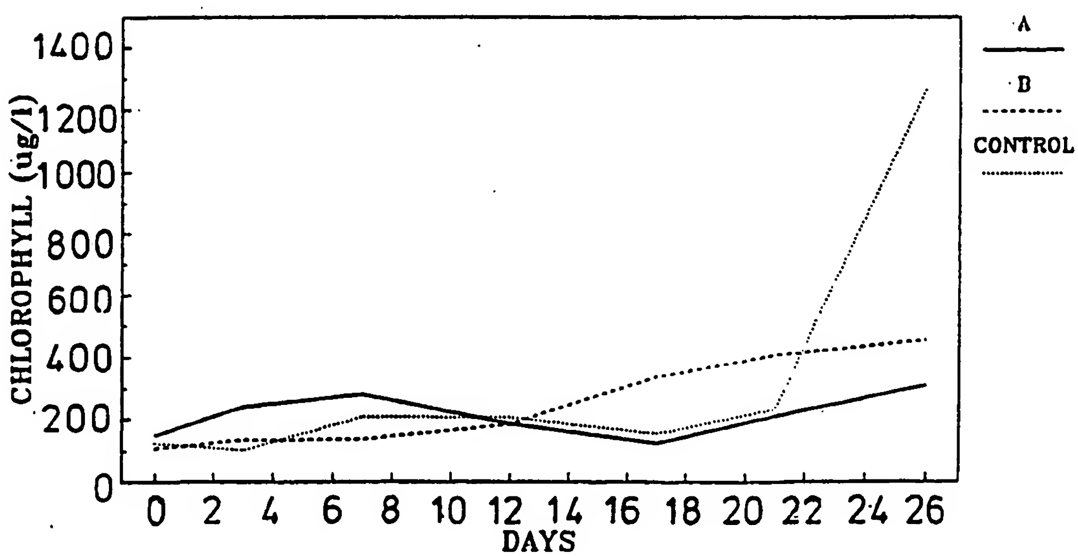
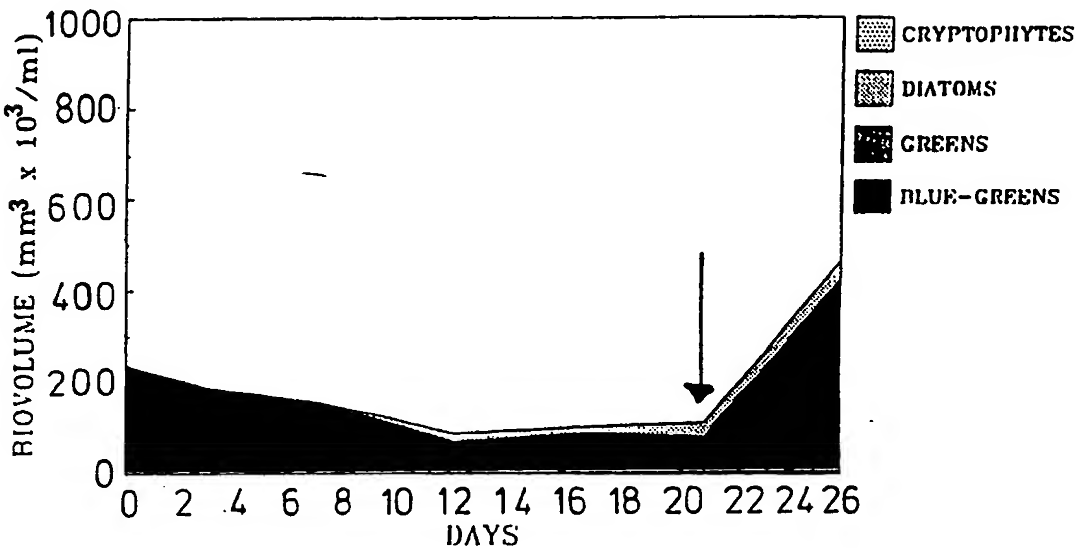


FIG.10

PHYTOPLANKTON
SALFORD QUAYS - OSCILLATORIA
pH RANGE: 6.5 - 7.0



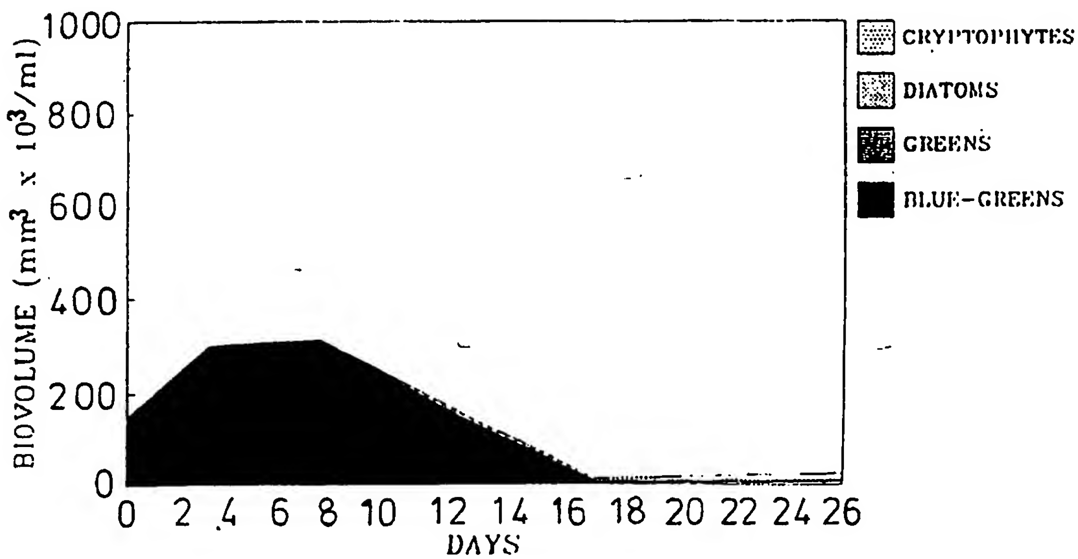


FIG 11

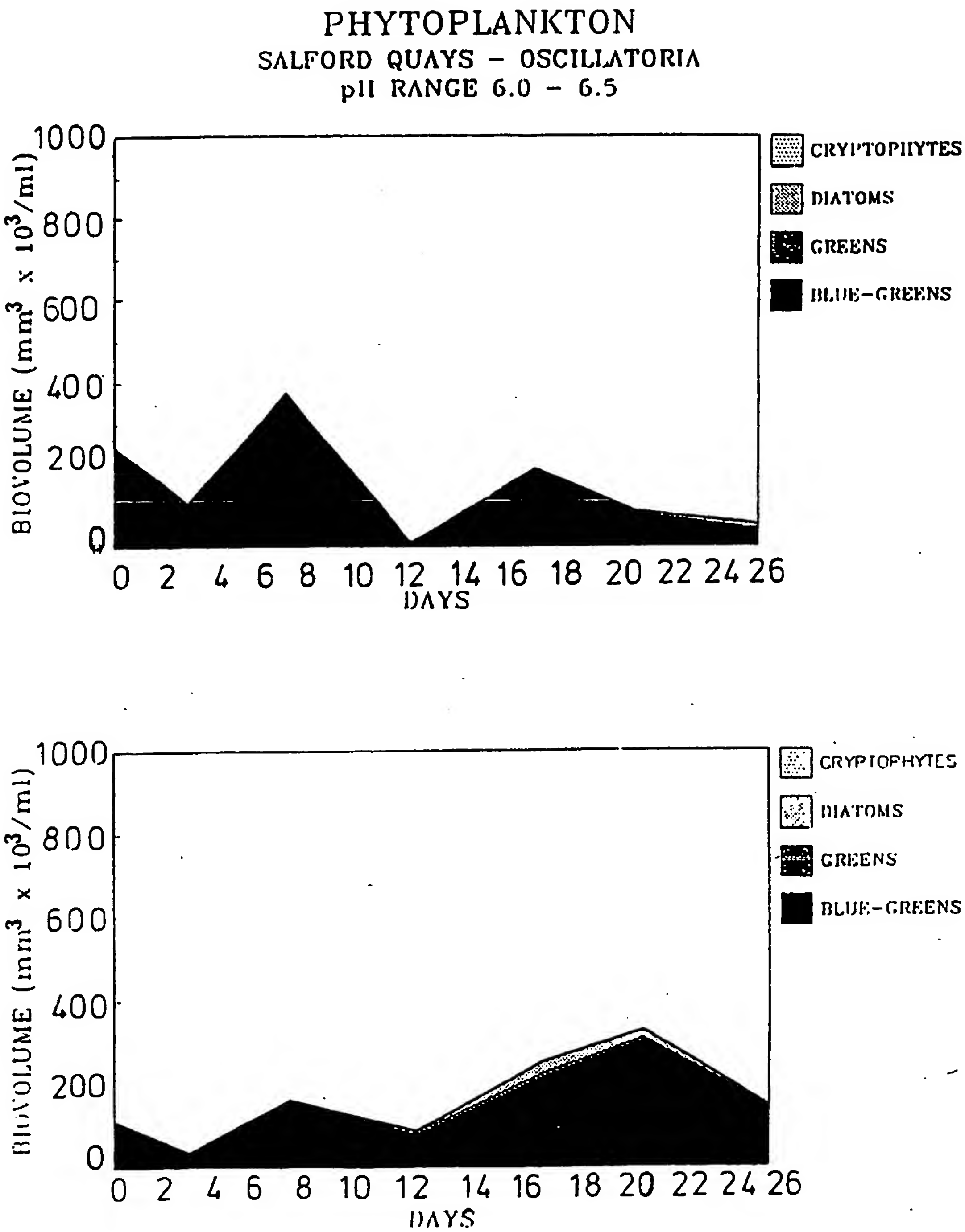

PHYTOPLANKTON
SALFORD QUAYS – OSCILLATORIA
pH RANGE 6.0 – 6.5
BIOVOLUME (mm^3 x 10^3/ml)
1000
800
600
400
200
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
DAYS
CRYPTOPHYTES
DIATOMS
GREENS
BLUE-GREENS
BIOVOLUME (mm^3 x 10^3/ml)
1000
800
600
400
200
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
DAYS
CRYPTOPHYTES
DIATOMS
GREENS
BLUE-GREENS

FIG.12

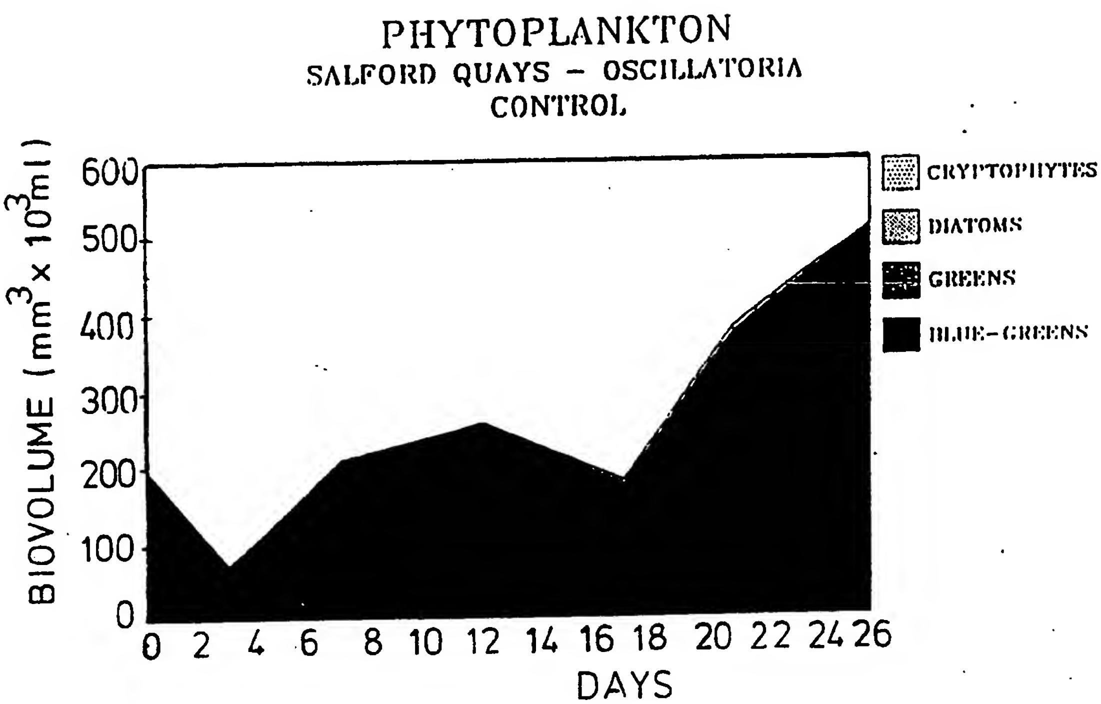

PHYTOPLANKTON
SALFORD QUAYS – OSCILLATORIA
CONTROL
BIOVOLUME (mm³ x 10³ml)
600
500
400
300
200
100
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
DAYS
CRYPTOPHYTES
DIATOMS
GREENS
BLUE-GREENS

FIG. 13

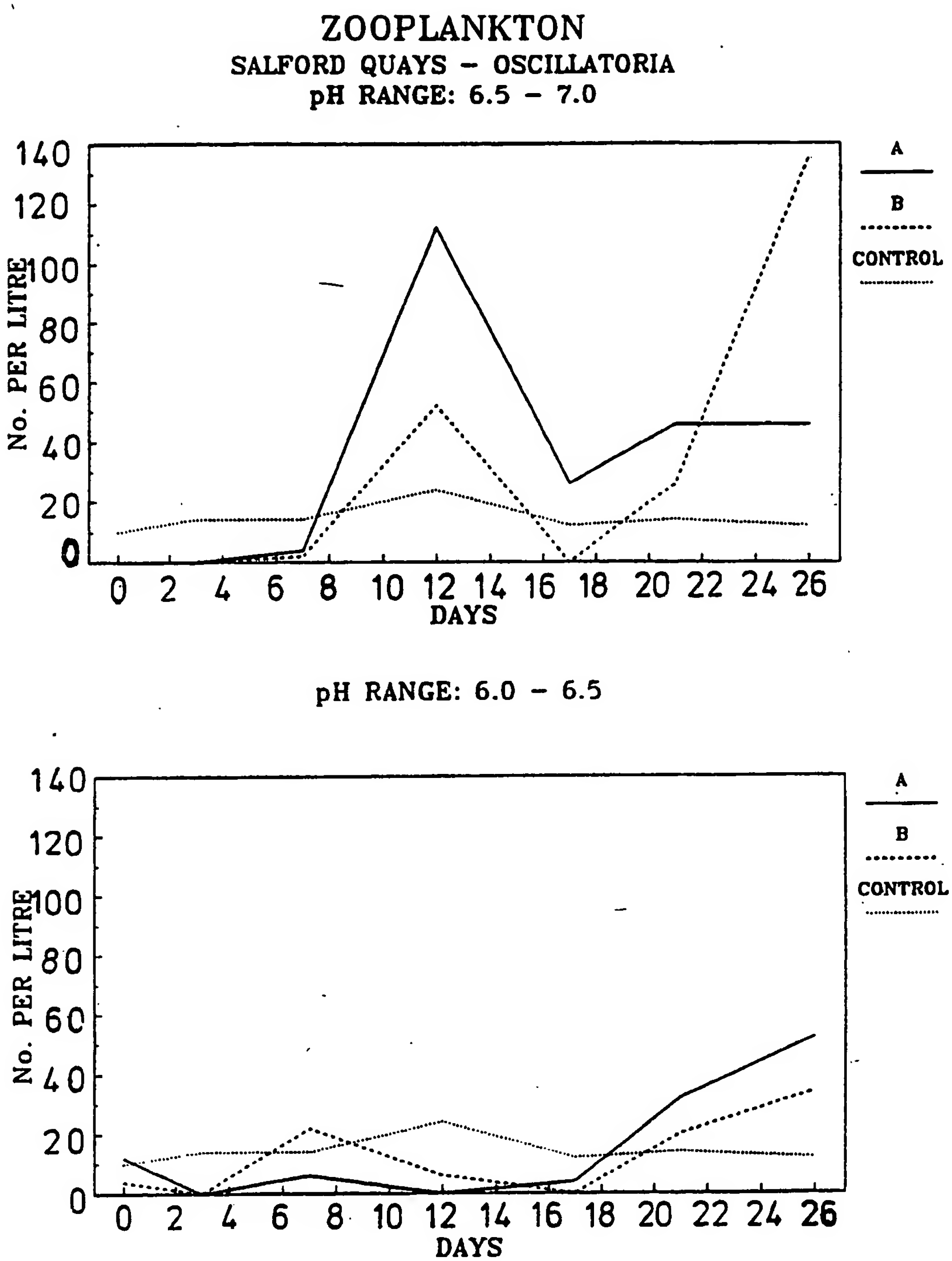
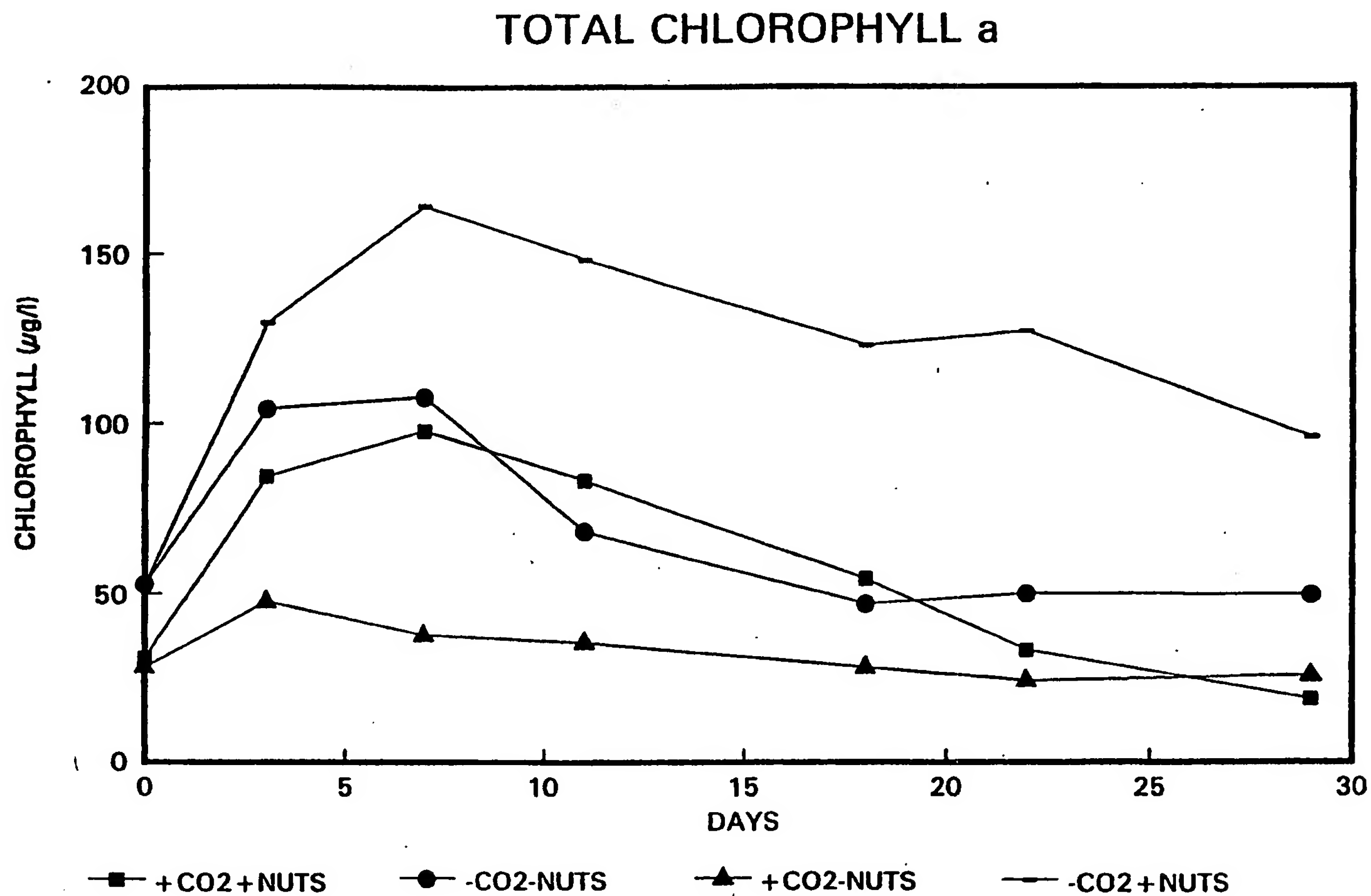
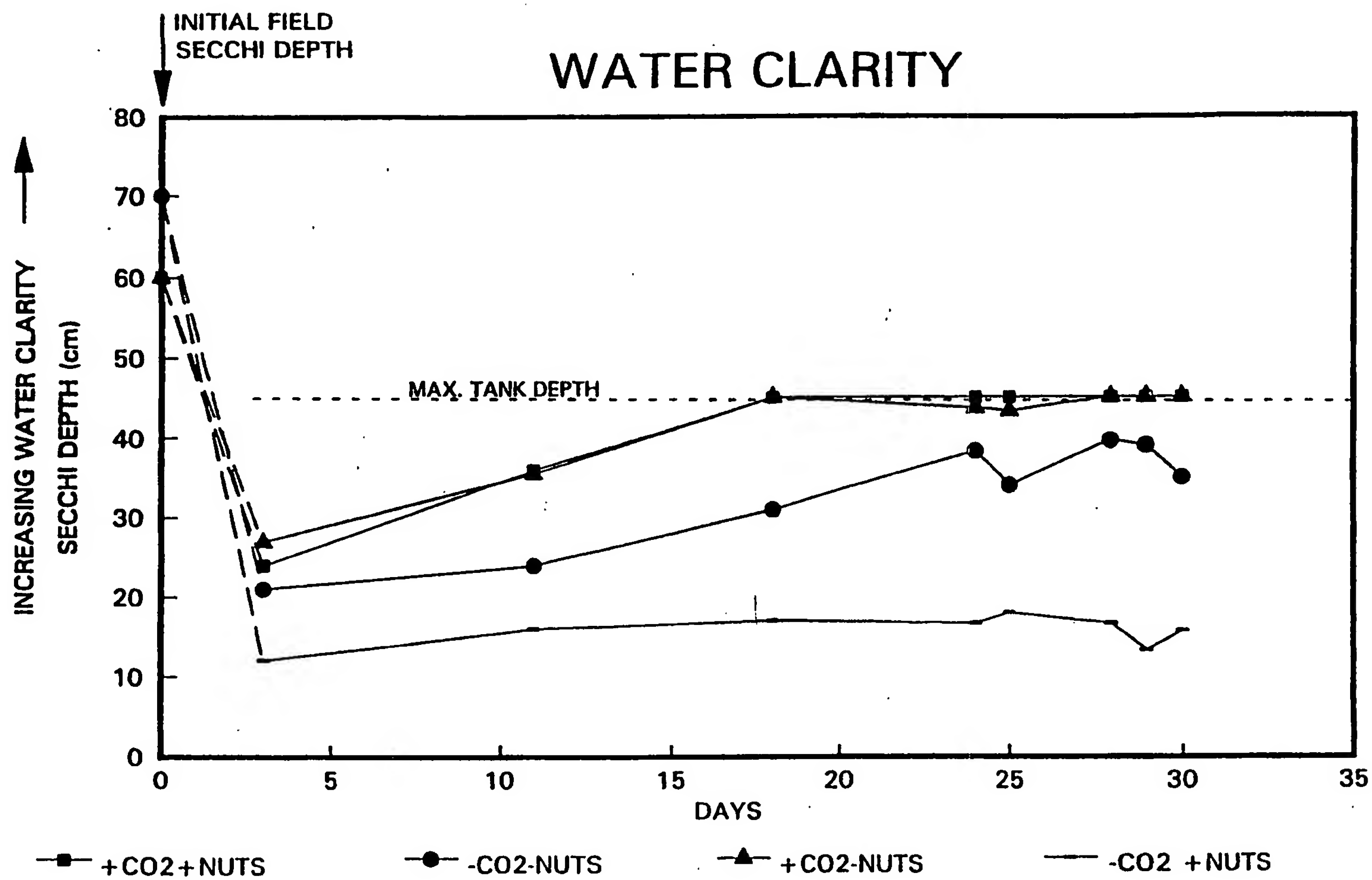

ZOOPLANKTON
SALFORD QUAYS - OSCILLATORIA
pH RANGE: 6.5 - 7.0
140
120
100
80
60
40
20
0
No. PER LITRE
0 2 4 6 8 10 12 14 16 18 20 22 24 26
DAYS
A
B
CONTROL
pH RANGE: 6.0 - 6.5
140
120
100
80
60
40
20
0
No. PER LITRE
0 2 4 6 8 10 12 14 16 18 20 22 24 26
DAYS
A
B
CONTROL

FIG.14



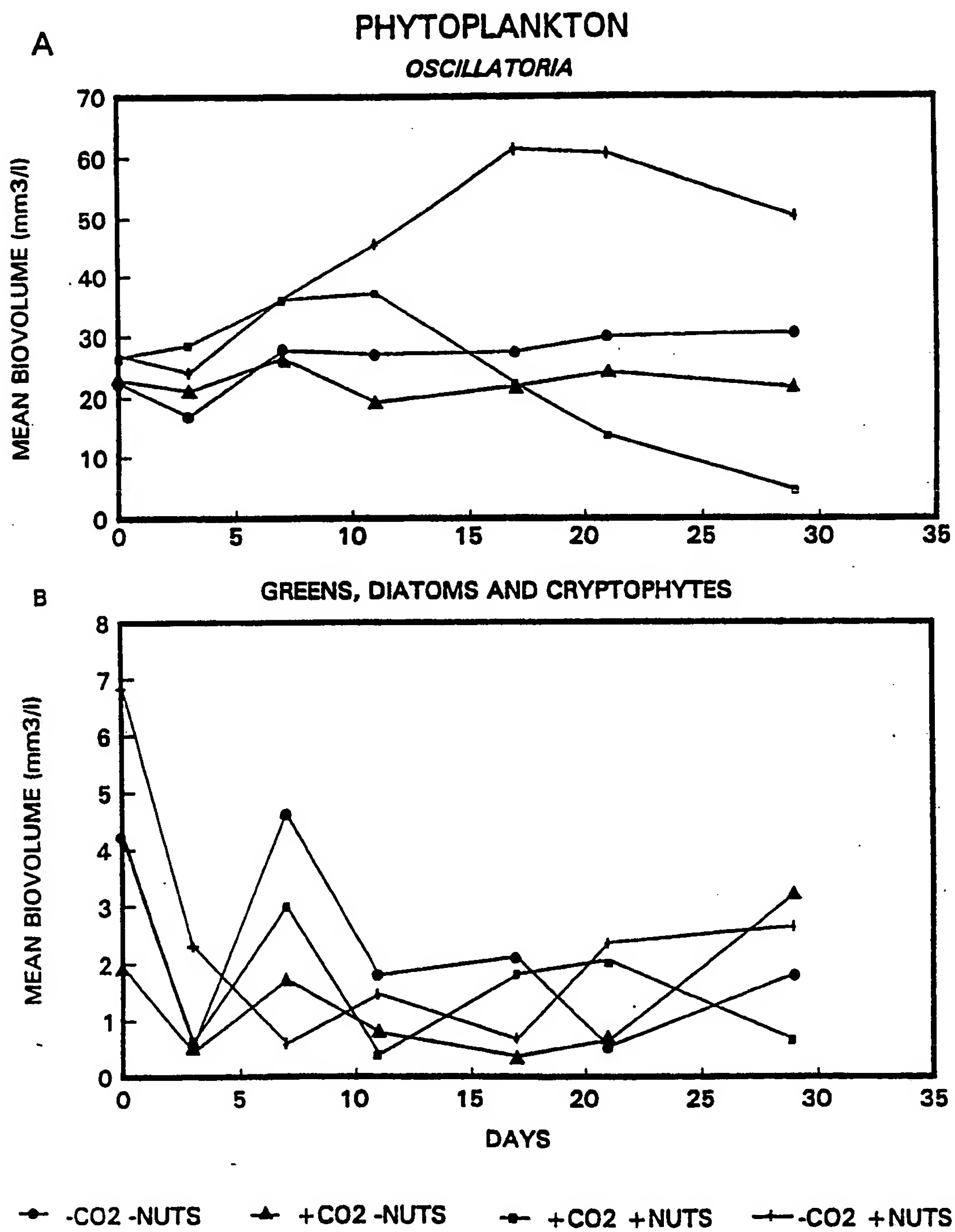
The effects of carbon dioxide and nutrient addition upon total chlorophyll a.

FIG.15



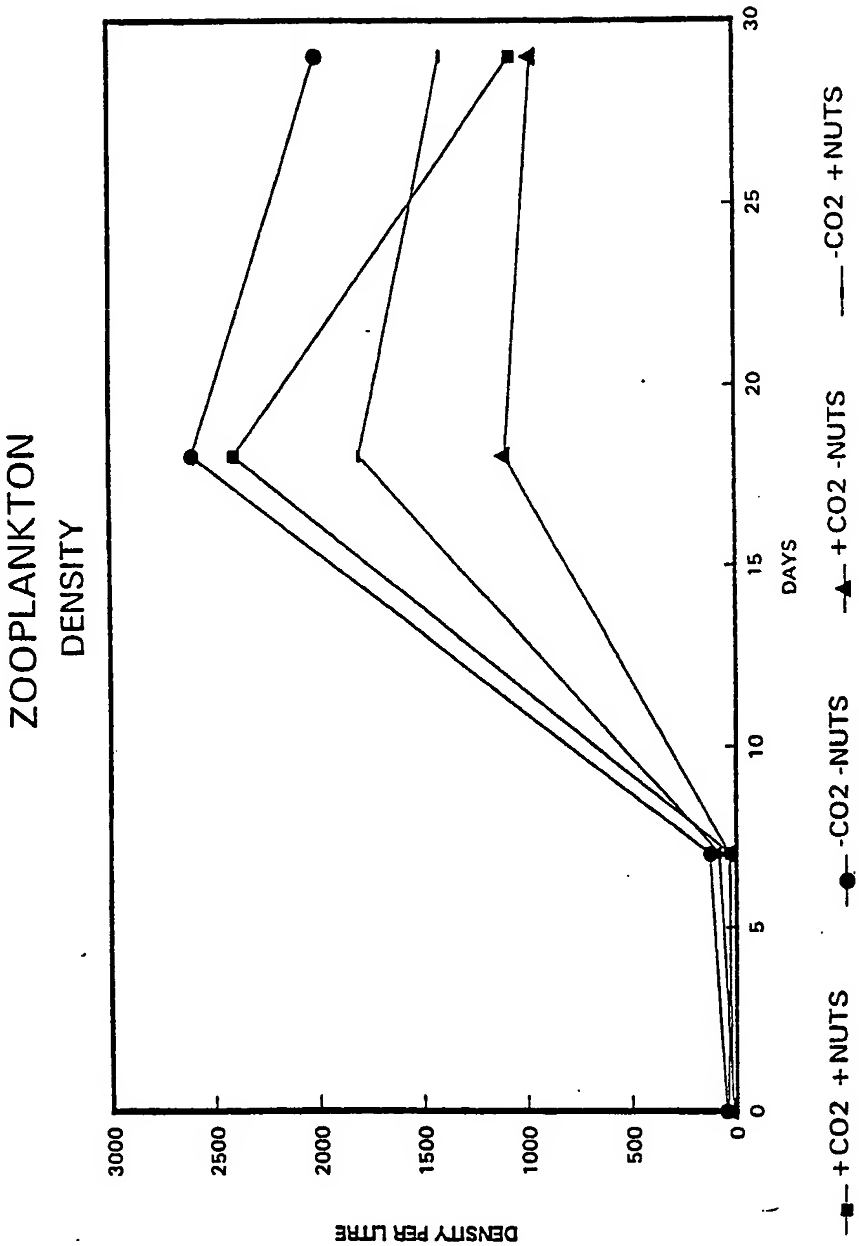
The effect of nutrient addition and carbon dioxide upon light extinction. Maximum water depth in each tank was 45cm. Arrow indicates initial water clarity in the field prior to exposure to laboratory conditions.

FIG.16



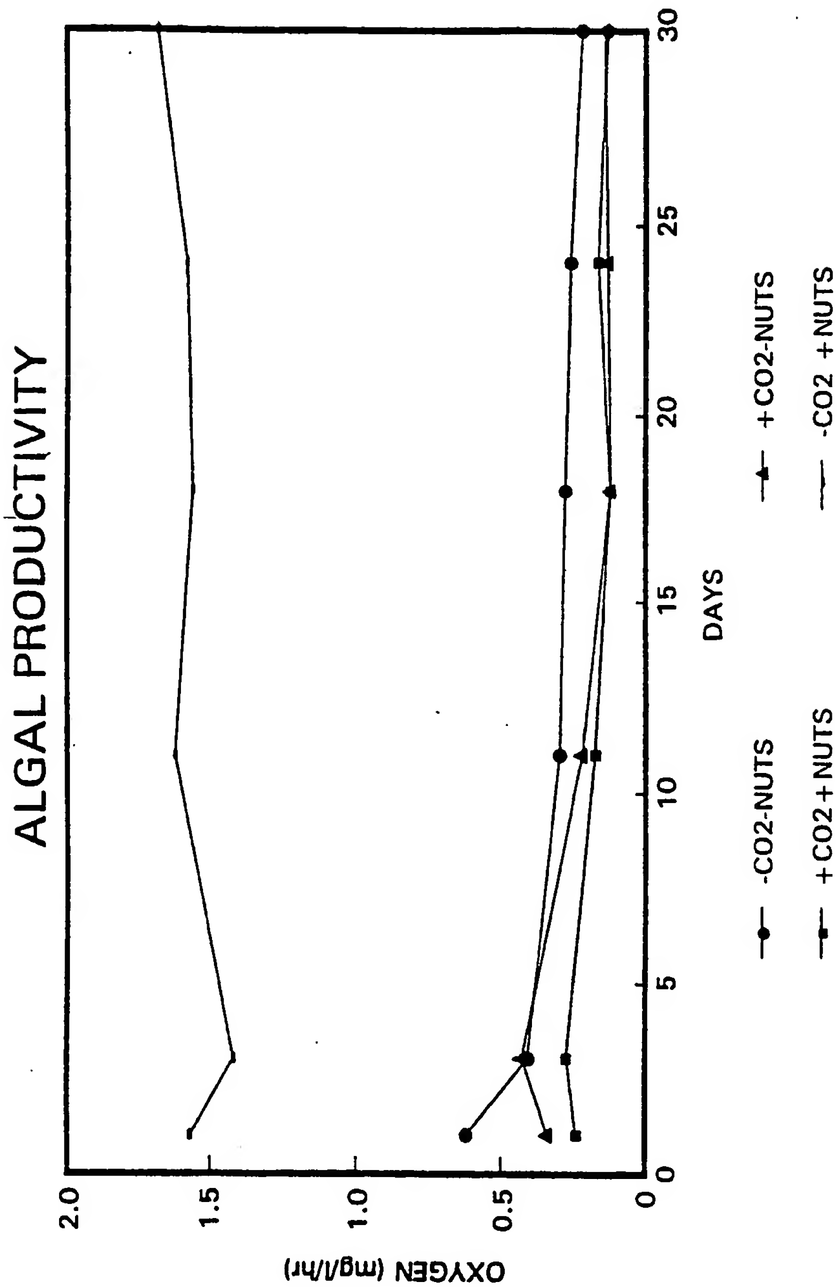
Mean Oscillatoria (A) and non-blue green algae (B) biovolumes after carbon dioxide and nutrient addition.

FIG.17



Changes in zooplankton density after addition of carbon dioxide and nutrients.

FIG.18



The influence of carbon dioxide and nutrient addition upon algal production.

FIG.19

International Application No PCT/GB 93/00772

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C02F1/66; C02F1/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C02F

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	US,A,3 756 220 (M.N.TEHRANI) 4 September 1973 cited in the application see the whole document -----	1,3-6,8, 9,11-13, 19-23, 25-28 2,21

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

IV. CERTIFICATION

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Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13 JULY 1993	29. 07. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	GONZALEZ Y ARIAS M.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300772
SA 73447

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 13/07/93
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-3756220	04-09-73	CA-A- 976281	14-10-75

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82